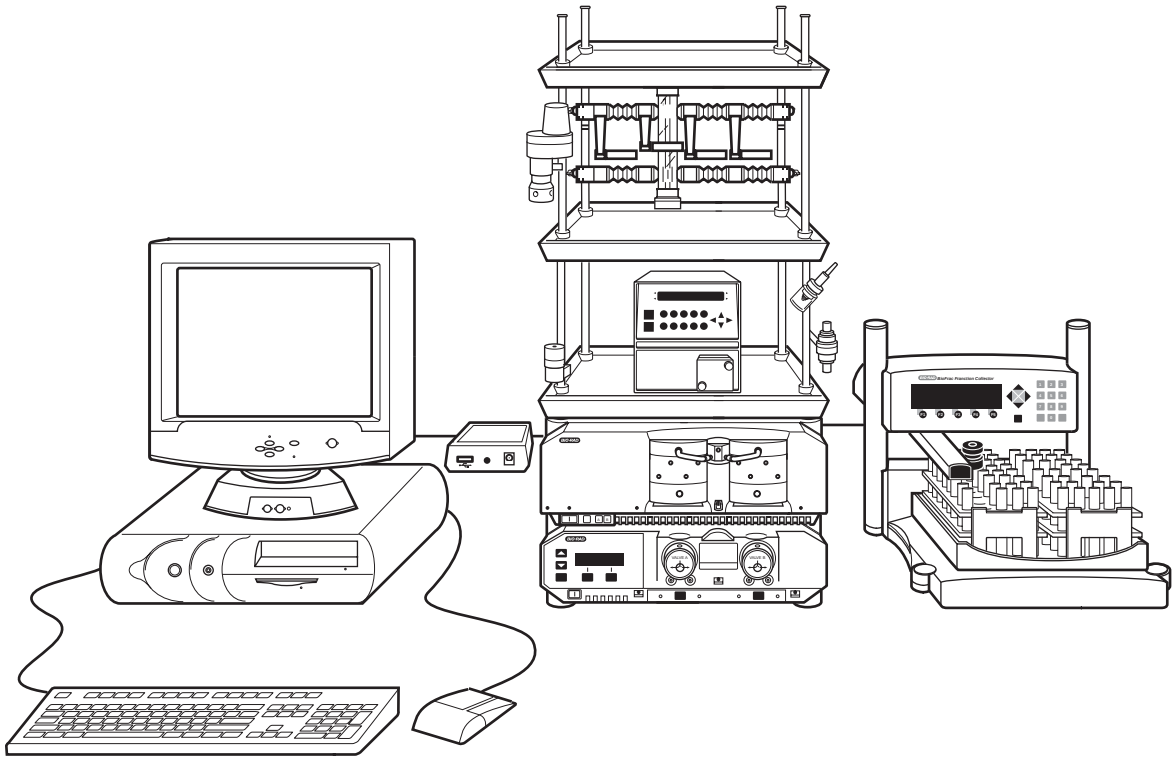

BioLogic DuoFlow™ CHROMATOGRAPHY SYSTEM

INSTRUCTION MANUAL

(BioLogic DuoFlow™ Software Version 5.0)

BIO-RAD

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1.0 INTRODUCTION

1.1 OVERVIEW

The BioLogic DuoFlow chromatography system is specifically designed for the high resolution purification of proteins, peptides, and other biomolecules where recovery of biological activity is of primary concern. The DuoFlow F10 pumphead operates at up to 20 ml/min and 3500 psi (233 bar, 23 MPa) when used with the Maximizer. The DuoFlow F40 pumphead operates at up to 80 ml/min and 1000 psi (66 bar, 6.6 MPa) when used with the Maximizer.

The BioLogic DuoFlow system software provides an easy-to-use graphic interface and menu-driven software for manual operation, system setup, method editing, and run operations. The system software may be run on any PC running Microsoft® Windows® 2000.

The flexible control architecture allows the seamless integration of a wide variety of configurations with other Bio-Rad and non-Bio-Rad components to meet your purification requirements.

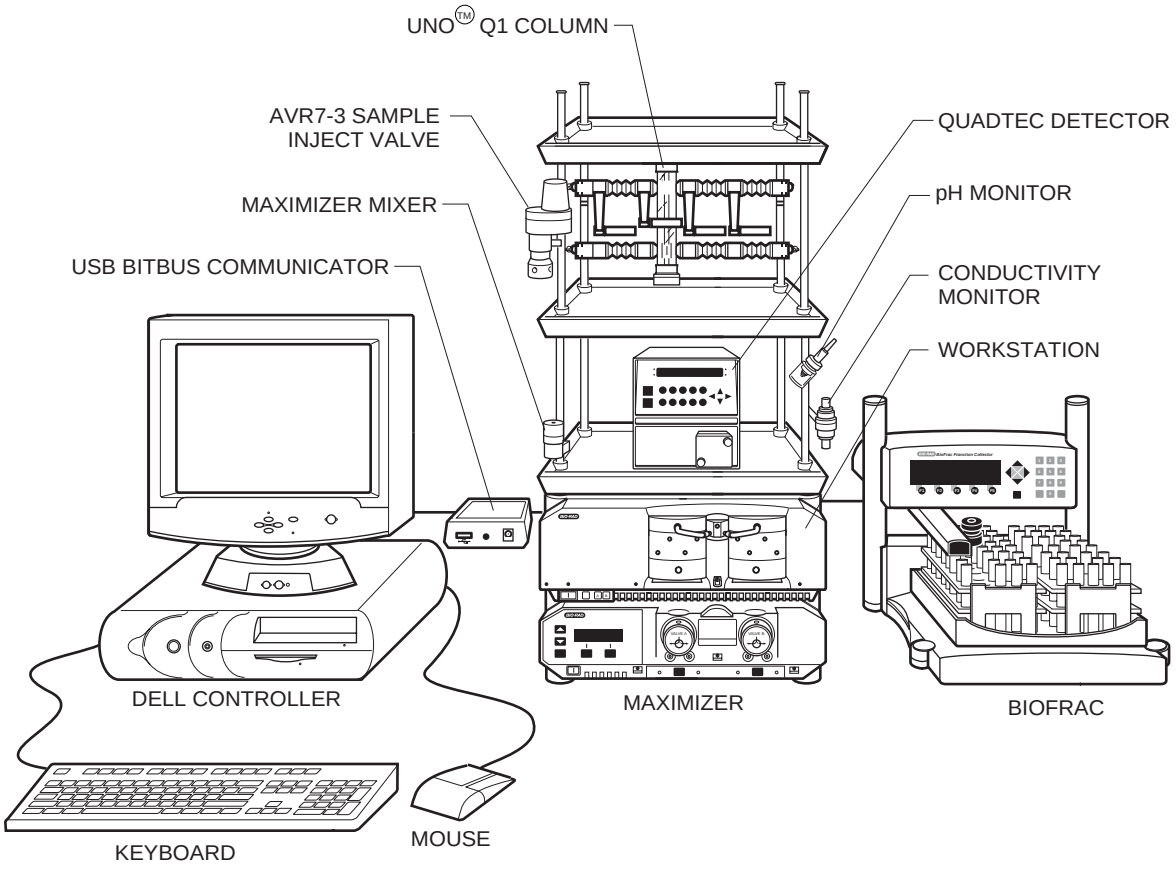


Figure 1-1. BioLogic DuoFlow System

1.2 FEATURES

BioLogic DuoFlow systems provide the following features:

- **Setup Flexibility.** A space saving modular design that is stackable and easily configured to meet your exact needs and fit into your desired bench space. Trays and vertical bars are moveable and removable. Horizontal bars can be placed in the optimal position for valves, columns, detectors, etc. The system fits easily into a cold box.
- **Modular** components provide an easy **upgrade path** to fit all applications and financial requirements. For example, the BioLogic DuoFlow™ Basic system may be purchased and upgraded to a BioLogic DuoFlow QuadTec™, BioLogic DuoFlow Maximizer or BioLogic DuoFlow Pathfinder system as needed. (See Section 1.4 for a description of the DuoFlow systems and upgrades.)
- **Intuitive, user-friendly software programming.** Users become an expert in only a few runs.
  - Four easy steps to set up new devices/instruments, create a new method, and start to run samples:

    Step 1. Browser, to enter new user name, project name and method name.

    Step 2. Setup, to specify devices required for the method.

    Step 3. Protocol, to enter sequential method steps.

    Step 4. Run, to inject sample and view the real time chromatogram.
  - Two easy steps, if you want to run a sample using a current Setup and Protocol:

    Step 1. Browser, to select a user and along with a method or Method Template.

    Step 2. Run, to inject sample and view real time chromatogram.
- **On screen Help**. Includes detailed information and a troubleshooting guide.
- **USB Bitbus Communicator**. The USB Bitbus communicator allows the BioLogic DuoFlow system to be controlled from any computer running Windows 2000 and BioLogic software 4.0 or above, over a USB port.
- **Buffer Blending.** Buffer Blending is a feature of the BioLogic DuoFlow Maximizer™ and Pathfinder systems that dynamically "Blends" the conjugate acid and base of a buffer with water and salt to produce a solution with a specific salt concentration and pH.
- **Buffer Editor.** The Buffer Editor is a feature used to create buffer systems for use in Buffer Blending experiments. Both single and multiple component buffers can be created.
- **Scouting.** This feature facilitates the optimization of a chromatography protocol for a specific target molecule. Scouting systematically increments a user selected variable and then performs a chromatography run at each increment. Variables that can be "Scouted" include: pH, %B, step duration, column, buffer, flow rate, sample and sample volume.
- **Method Templates.** Includes ready-to-run chromatography protocols for a variety of experiment types including: affinity, chromatofocusing, hydroxyapatite, hydrophobic interaction, ion exchange and gel filtration chromatography.
- **pH Monitor.** The BioLogic pH monitor allows direct pH monitoring during a run. It is included as part of the DuoFlow Maximizer and Pathfinder systems and is available as an option for all other system configurations. The pH monitor consists of a Calomel Tris compatible electrode in a PEEK biocompatible flow cell.
- **Flow Rate Flexibility.** The DuoFlow Workstation has two pump head options (F10 and F40) that permit a wide range of flow rates (0.01 ml/min up to 80 ml/min).
- **Detection Flexibility**
  - UV detector with fixed 254 nm and 280 nm filters, long life mercury lamp, and additional drop-in expansion filters available.
  - UV detector can be expanded to 214 nm filter with zinc lamp.
  - QuadTec UV/Vis detector analyzes samples simultaneously at 4 different wavelengths from 190-370 nm with a deuterium lamp or 370-790 with a halogen lamp.

  - Third party detectors, such as refractive index or fluorescence, may be utilized with the DuoFlow systems via a Signal Import Module (SIM) or Maximizer.
- **Conductivity Monitor.** Monitors salt concentration to assure reliable gradient formation and pump function.
- **Fraction Collection.** The DuoFlow system supports a wide variety of fraction collection options including: Collect All, Threshold Collection, Collection Windows and Threshold & Collection Windows. Both Above Threshold and Below Threshold collection are supported. The software also supports tube numbering by Rack & Tube # and Rack & Grid #.
- **Multiple Valve Capabilities**
  - Workstation provides connection for 3 low pressure and 3 high pressure valves.
  - Workstation with the addition of the Maximizer doubles the capacity to 6 low pressure and 6 high pressure valves.
- **Starter Kit and UNO Q1 Anion Exchange Column.** Includes the necessary reagents, protein sample and columns for running an anion exchange chromatography experiment. The kit includes easy to follow, tutorial style instructions for the first time user.
- **IQ/OQ Protocols**. Validation protocols are available or can be performed by certified Bio-Rad service engineers.

1.3 UNPACKING

When you receive the BioLogic DuoFlow system, carefully inspect the shipping containers for any damage which may have occurred in shipping. Severe damage to a container may indicate damage to its contents. If you suspect damage to the contents, immediately file a claim with the carrier in accordance with their instructions before contacting Bio-Rad Laboratories.

Caution
Lift items from the bottom as you remove them from their containers!

Open each of the shipping cartons and lift the contents out of its packing. Check the contents of each box against the supplied packing list. Remove the plastic bag from each unit and inspect the unit for external damage. If any part is missing or damaged, contact Bio-Rad Laboratories immediately.

Bio-Rad ships DuoFlow systems in a number of different configurations, each with its own catalog number. These systems are described in the following table. Because of its modular design, any of these systems can be upgraded any time simply by adding system options.

1.4 SYSTEM CONFIGURATIONS

The BioLogic DuoFlow is available in the following system configurations. Each system configuration is identified by its name, its standard components and devices, and optional components and devices that may be ordered separately for use with the system.

Catalog Number	BioLogic DuoFlow System Guide S = Standard U = Upgradable	F10 Workstation	F10 Pump Kit	F40 Workstation	F40 Pump Kit	BioFrac Fraction Collector	QuadTec UV/Vis Detector	Maximizer (incl pH)	pH Monitor
760-0037 760-0036 760-0038	DuoFlow Basic System 100/120 V DuoFlow Basic System Japan and Korea DuoFlow Basic System 220/240 V *10 ml/min flow rate to 3500 psi* *254/280 nm detection*	S	S	U	U	U	U	U	U
760-0047 760-0046 760-0048	DuoFlow Standard System 100/120 V DuoFlow Standard System Japan/Korea DuoFlow Standard System 220/240 V *10 ml/min flow rate to 3500 psi* *254/280 nm detection* *Fraction collection*	S	S	U	U	S	U	U	U
760-1137 760-1136 760-1148	DuoFlow QuadTec Basic System 100/120 V DuoFlow QuadTec Basic System Japan/Korea DuoFlow QuadTec Basic System 220/240 V *10 ml/min flow rate to 3500 psi* *UV/Vis detection with 4 simultaneous wavelengths*	S	S	U	U	U	S	U	U
760-1147 760-1146 760-1148	DuoFlow QuadTec Standard System 100/120 V DuoFlow QuadTec Standard System Japan/Korea DuoFlow QuadTec Standard System 220/240 V *10 ml/min flow rate to 3500 psi* *UV/Vis detection with 4 simultaneous wavelengths* *Fraction collection*	S	S	U	U	S	S	U	U
760-2237 760-2236 760-2238	DuoFlow Maximizer 20 System 100/120 V DuoFlow Maximizer 20 System Japan/Korea DuoFlow Maximizer 20 System 220/240 V *20 ml/min flow rate to 3500 psi* *254/280 nm detection* *Buffer blending* *Fraction collection*	S	S	U	U	S	U	S	S
760-2247 760-2246 760-2248	DuoFlow Maximizer 80 System 100/120V DuoFlow Maximizer 80 System Japan/Korea DuoFlow Maximizer 80 System 220/240 V *80 ml/min flow rate to 1000 psi* *254/280 nm detector* *Buffer blending* *pH monitoring* *Fraction collection*	U	U	S	S	S	U	S	S
760-2257 760-2256 760-2258	DuoFlow Pathfinder 20 System 100/120 V DuoFlow Pathfinder 20 System Japan/Korea DuoFlow Pathfinder 20 System 220/240 V *20 ml/min flow rate to 3500 psi* *UV/Vis detection with 4 simultaneous wavelengths* *Buffer blending* *pH monitoring* *Fraction collection*	S	S	U	U	S	S	S	S
760-2267 760-2266 760-2268	DuoFlow Pathfinder 80 System 100/120 V DuoFlow Pathfinder 80 System Japan/Korea DuoFlow Pathfinder 80 System 220/240 V *80 ml/min flow rate to 1000 psi* *UV/Vis detection with 4 simultaneous wavelengths* *Buffer blending* *pH monitoring* *Fraction collection*	U	U	S	S	S	S	S	S

1.5 QUICK START PROCEDURE

The general procedure used to create and run a chromatography experiment on a DuoFlow system is described below.

1. Install the required devices and instruments on the system (see Figures 3-4 and 5-5 for cable connections and 4-1 and 4-3 for plumbing connections).
2. Flush all plumbing with DDI H_2O to ensure that the system is clean and free of air and then prime the pumps with starting buffer. See Chapter 4 for more detail.
3. Attach a column and set the pressure limits in the Manual screen. The high limit should be less than or equal to the pressure limit for the column.
4. Equilibrate the column and system with starting buffer. System equilibration is controlled from the Manual screen (see Section 7.1).
5. Create a new method in the Browser.
   a. Start the Browser using the **Browser** button on the tool bar and then select or create a user and project. Refer to Chapter 6 for more information on the Browser screen.
   b. Use the **New** or **New/New User** option in the Browser tools, on the left side of the screen, to create a new user and enter a username.
   c. Use the **New** and **New Method** option in the Browser tools to create and name a new method. Click **OK** to proceed to the hardware Setup screen. Alternatively, check the Use Method Templates box, select a method template and press **OK**.
6. In the Device Setup screen, select the devices that are connected to the system. Select the **File/Save Setup** menu item and save the device setup (check the Default Setup box if this is the default setup). Refer to Section 7.2 for more information about the Device Setup screen.
7. Start the Protocol Editor using the tool bar Protocol Editor button. Use the protocol screen Add Step tools to create a new method as illustrated with below for an ion exchange protocol. See Chapter 7 for more information.
   a. Add an **Isocratic Flow** step to equilibrate the column and enter the required flow rate, step size and %B. The parameters entered here will automatically appear in the next step, but can be changed at any time.
   b. Add a **Zero Baseline** step to zero the selected detector prior to sample injection.
   c. Add a **Load Inject Sample** step and then enter the sample inject volume and flow rate. If a static injection loop is used with an AVR7-3 Sample Inject Valve select static loop. For information about the other injection options see Chapter 8.
   d. Add an **Isocratic Flow** step to wash the column and enter the required flow rate, step size and %B.
   e. Add a **Linear Gradient** step to elute the column and enter the required flow rate, step size, initial %B and final %B.
   f. Add an **Isocratic Flow** step to clean the column and enter the required flow rate step size and %B (usually 100%). This ensures that the entire sample is removed from the column.
   g. Add an **Isocratic Flow** step to re-equilibrate the column and enter the required flow rate step size and %B (see step a, above).
   h. Add a **Fraction Collection** step to specify how fractions are to be collected for the experiment. Enter the collection technique (e.g. collect all, threshold, etc), fraction size and any required threshold or collection windows parameters. Note that the dialog displays the number of tubes required for the currently defined protocol.
8. Press the **New Run** button to create a new run, enter a run name and open the Run screen.
9. Set the appropriate pressure limits for the column being used and then press Start on the system tool bar. Refer to Chapter 7.4 for more information about the Run screen.

2.0 DESCRIPTION OF SYSTEM COMPONENTS

The DuoFlow's modular design supports many types of system components, and allows for a wide variety of system configurations. This section discusses in detail the function of each component and its connection to the system.

- Dell PC Computer/Controller and USB Bitbus Communicator (Section 2.1)
- Workstation (Section 2.2)
- Maximizer (Section 2.3)
- Mixers: MX-1 and Maximizer mixers: (Section 2.4)
- Detection Systems: UV detector, Conductivity monitor, pH monitor, and QuadTec detector: (Section 2.5)
- Valves: AVR7-3, AVR9-8, SV5-4, and SVT3-2 valves: (Section 2.6)
- Fraction Collectors: BioFrac, Model 2128, and Model 2110: (Section 2.7)
- Sample Loading Options: DynaLoop, EP-1 Econo pump, and EGP Econo Gradient Pump: (Section 2.8)
- System peripherals: (Section 2.9)
- Columns and Column Fittings: (Section 2.10)

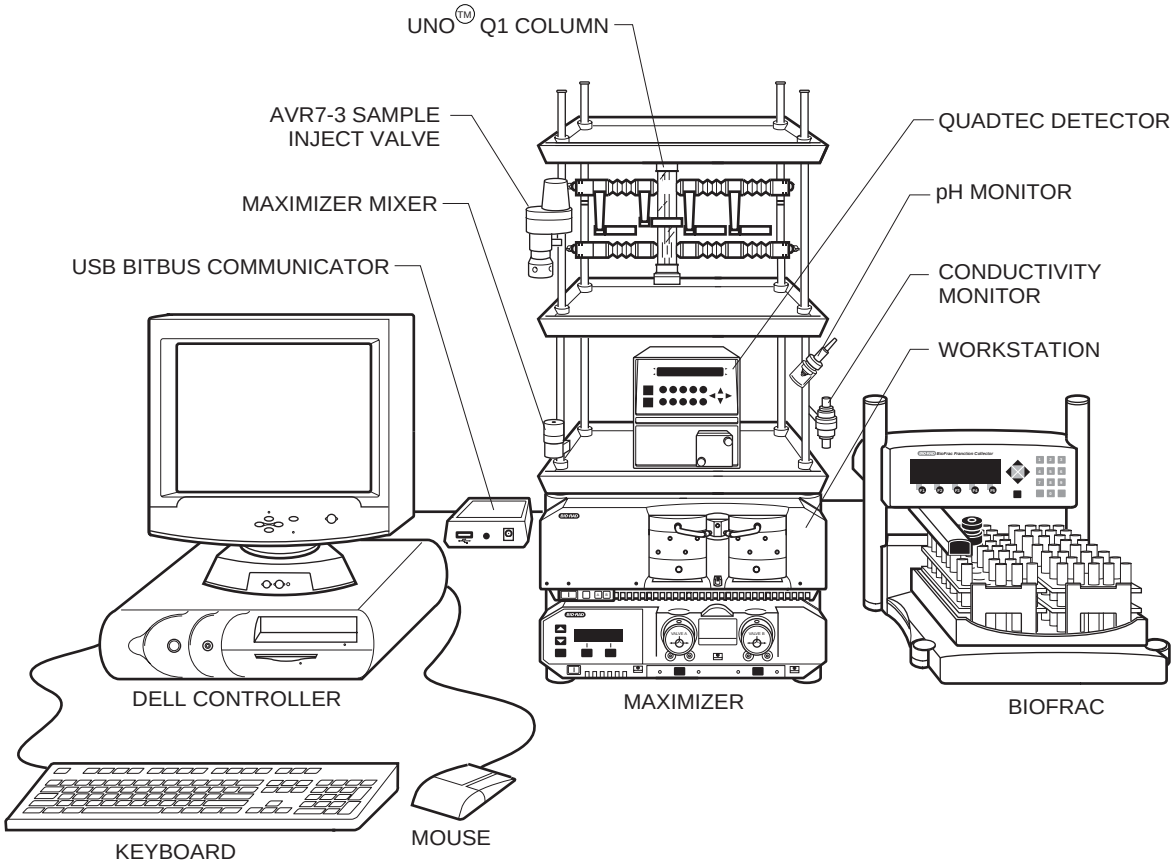


Figure 2-1. BioLogic DuoFlow Pathfinder System Components

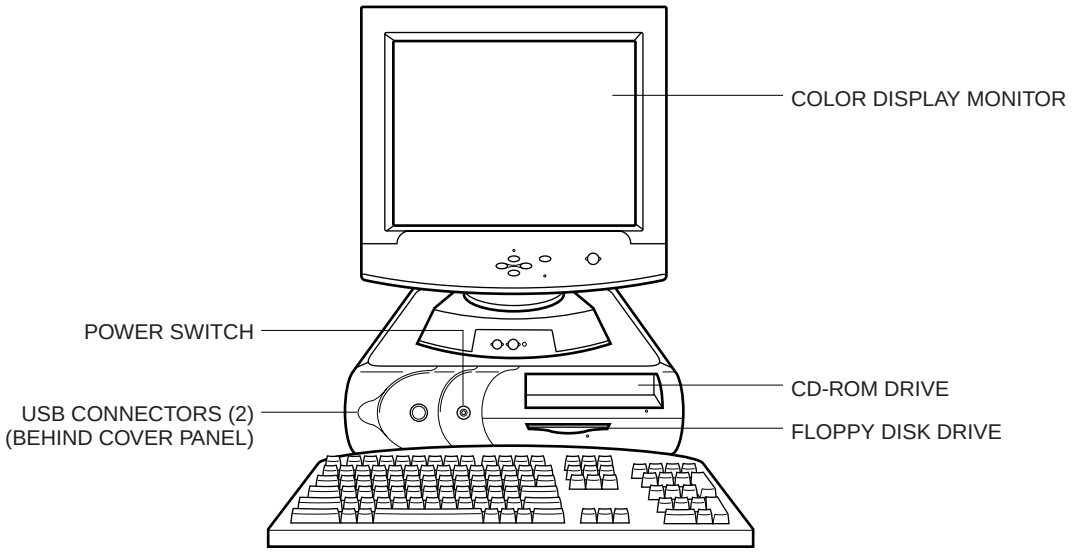
2.1 CONTROLLER AND USB BITBUS COMMUNICATOR

The DuoFlow system is controlled by a PC computer, referred to throughout this document as the Controller. The Dell Controller available from Bio-Rad includes a color display monitor, a keyboard, a mouse device, a CD-ROM drive, and a floppy disk drive. The Controller communicates with the workstation and other external devices through its USB connector. The USB Bitbus Communicator serves as the link between the Controller's USB port and the DuoFlow's instrument bus. This link allows the Controller to control the Maximizer and Workstation, as well as any devices connected to the Maximizer or Workstation, such as automatic valves, UV detector and conductivity monitor, and peripheral instruments such as the BioFrac fraction collector, the QuadTec UV/Vis detector, the Model EP-1 Econo pump, the Econo Gradient Pump (EGP), and Signal Import Modules (SIM).

2.1.1 Controller

The Controller runs the BioLogic DuoFlow software (version 4.0 or higher) on a Windows® 2000 operating system. From the Controller you can set up and run methods, perform simple data analysis, and store method and run data. The following tables show the key features on the Dell computer provided by Bio-Rad.

Table 2-1.
Front View of the Dell PC Computer as the DuoFlow Controller

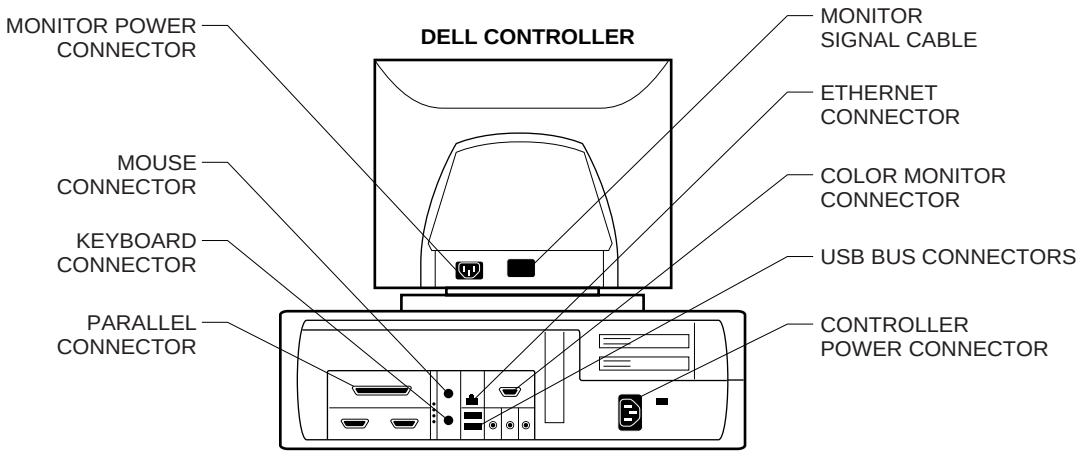


Feature	Description
Power Switch	Turns on/off the Controller and monitor.
Floppy Disk Drive	To backup methods to - and restore methods from - a floppy disk. Press the button next to the drive slot to manually eject a floppy disk from the drive.

Table 2-1. (continued)
Front View of a Dell PC Computer as the DuoFlow Controller

Feature	Description
CD ROM Drive	To load updates of the BioLogic DuoFlow operating software. To open the drive, press the button on the front of the drive.
Keyboard, Mouse, & Function Keys    	The keyboard and mouse interface devices control the system. They are standard PC compatible input devices. The keyboard includes the following special function keys: **Hold until Keypress:** To start a method that is on Hold during a run when a method includes a “Hold until Keypress” step. **Help:** Displays the Help menu for the currently displayed screen. **Esc:** Functions as an alternative to the Cancel selection in a Dialog box. **Alt:** Some system commands can be executed either by selecting them from a drop-down menu or by holding down the **Alt** key and then pressing the appropriate character key.
USB Connectors	These connect to the USB Bitbus Communicator, which in turn connects to the instrument bus, and allows components of the BioLogic DuoFlow system to communicate with the Controller. Components connect to the instrument bus in a “daisy-chain” and are recognized when the system is switched on. Even when one component is switched off, other components “daisy-chained” to the system can be controlled by the Controller.

Table 2-2.
Rear View of the Dell PC Computer as the DuoFlow Controller



Connector	Description
	USB connectors: These connect to the USB Bitbus Communicator, which in turn connects to the instrument bus, and allows components of the BioLogic DuoFlow system to communicate with the Controller. Components connect to the instrument bus in a “daisy-chain” and are recognized when the system is switched on. Even when one component is switched off, other components “daisy-chained” to the system can be controlled by the Controller.
	Monitor connector: To connect the color monitor to the Controller.
	Keyboard connector: To connect the keyboard to the Controller.
	Mouse connector: To connect the mouse to the Controller.
	Parallel connector: Devices designed for connection to the parallel port include printers and external storage devices. Refer to Windows® 2000 and/or the device documentation for installation instructions. (Some printer drivers are pre-installed on the Controller.)
	Power connectors: To connect the power cable.

2.1.2 USB Bitbus Communicator

The USB Bitbus Communicator is used to connect the Controller to DuoFlow system instrument bus and to supply power to the instrument bus when a Signal Import Module (SIM) is used

Table 2-3.
USB Bitbus Communicator

FRONT VIEW **REAR VIEW**

Feature	Description
USB	Used to connect to the Controller USB port by way of a USB cable (catalog number 760-2032).
ON LED	Indicates that there is power to the USB Bitbus Communicator.
POWER	Is used to supply power to the USB Bitbus Communicator when a Signal Import Module (SIM) is connected to DuoFlow instrument bus. The universal AC/DC inline adapter (catalog number 760-2034) must be used to supply the power. Use of other power adapters may damage the USB Bitbus Communicator.
PWR SELCT	Used to select whether power for the USB Bitbus Communicator is drawn from the Controller (INT) or the AC/DC inline adapter (EXT). An external power source must be used if a Signal Import Module (SIM) is connected to the instrument bus otherwise it is not required.
BUS PWR LED	Indicates that the instrument bus is receiving power from an external power source. The light is off when the PWR SELCT switch is set to INT. The light will turn on when the USB Bitbus Communicator is switched to EXT and power is being received.
INSTR BUS	Used to connect the USB BitBus Communicator to the DuoFlow instrument bus.

2.2 WORKSTATION

The Workstation contains the following:

- Dual pumpheads, each consists of two biocompatible dual piston pumpheads. A built-in pressure transducer is located on the workstation at the pump outlet. The pressure transducer measures system pressure, which is displayed on the lower status bar of the software Manual and Run screens. Purge and Pause buttons (Purge A, Purge B, and Pause) are present on the front of the Workstation.

  There are two types of pumpheads; the F10 and the F40. All DuoFlow systems have F10 pumpheads, except DuoFlow Maximizer 80 and DuoFlow Pathfinder 80 systems that have F40 pumps.

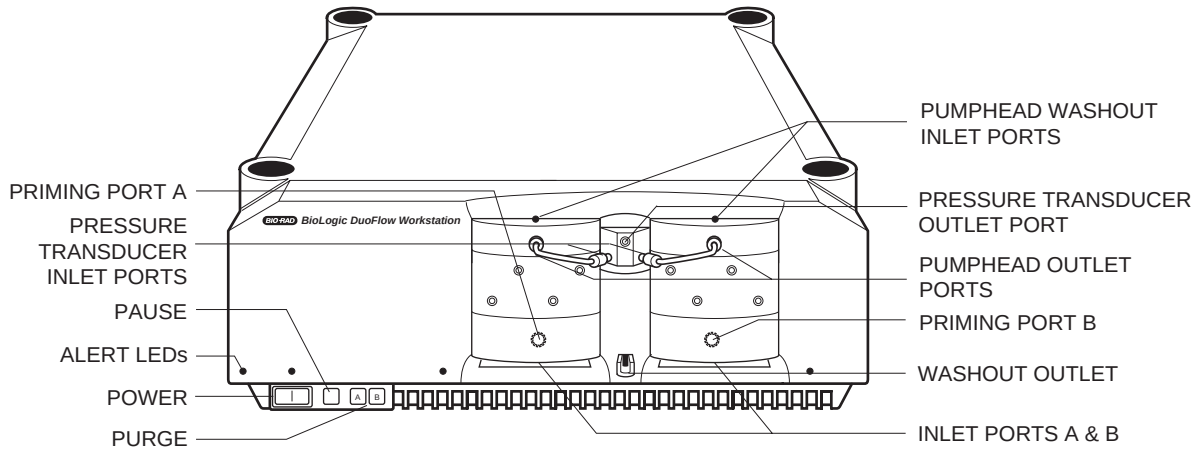
F10 and F40 pumphead kits are available to easily convert an F10 Workstation to F40 and vise versa. (See Section 2.9.7)

Table 2-4.
F10 and F40 Pumphead Flow Rates

Pumphead	Flow Rate: Isocratic and Gradient Mode	Flow Rate with Maximizer: High Flow Non-blending	Flow Rate with Maximizer: Buffer Blending Mode
F10	0.01 - 10 ml/min 3500 psi (233 bar, 23 MPa)	0.02 - 20 ml/min 3500 psi (233 bar, 23 MPa)	0.5 - 20 ml/min 3500 psi (233 bar, 23 MPa)
F40	0.5 - 40 ml/min 1000 psi (66 bar, 6.6 MPa)	1.0 - 80 ml/min 1000 psi (66 bar, 6.6 MPa)	1.0 - 80 ml/min 1000 psi (66 bar, 6.6 MPa)

- The Workstation houses the control circuitry for the Workstation pumps, MX-1 mixer, UV detector, Conductivity monitor, and system valves (low pressure solenoid and automated high pressure inject and select valves). Connectors on the rear panel provide inputs for valves and detectors, as well as output to a chart recorder for UV and conductivity data at 1 V Full Scale and pen up/down, start/stop control.
  If a Maximizer is being used, connect devices to it to connect the mixer, Conductivity monitor, and pH monitor to it, rather than to the Workstation.
- An AUX combicon connector used for (a) controlling fraction advances of the Model 2110 and generic fraction collectors, (b) receiving an open/closed signal from a device such as a manual inject valve, and (c) starting and stopping the Model EP-1 Econo pump for sample loading.
  If a Maximizer is being used, connect these devices to its AUX connector, rather than to the Workstation.
- Power supply for the Workstation electronics as well as all devices connected to and controlled by the Workstation.
- Output power connectors to the UV lamp and a Model 1327 chart recorder.
- Two instrument bus (phone-type) connectors.

Table 2-5.
Workstation Front Panel Controls

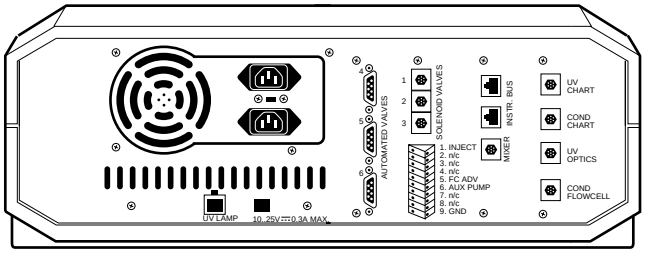


Feature	Description
Power Button	Turns power on/off to the Workstation and to the components connected to it.
Pause Button	Stops the Workstation pumps and pauses a running method. When it is pressed, the status LEDs for pumps A and B change from green to flashing red. To resume the run, • Press the button again, to restarts the pumps and the method, or • Press the **Continue** button in the Run screen, or • Press the **START** button in the Manual screen.
Purge A & B Buttons	Before you press either purge button switch the AVR7-3 inject valve to the Purge position in the Manual screen, so that the column is not exposed to high pressures and flow rates. The purge buttons flush the tubing lines with the solution connected to pumps A and B. When the buttons are pressed, the green LEDs for pumps A and B flash. To stop the flow, press the purge buttons again and the LEDs go off. These buttons do not operate when the system is running a method. When the purge buttons are pressed, the pumpheads run at their (default) maximum flow (F10 purge rate is 10.0 ml/min or 20 ml/min when used with the Maximizer; F40 purge rate is 40 ml/min or 80 ml/min when used with the Maximizer.) To alter the default purge flow rate, select Manual Setup from the Options drop-down menu.
Alert Light	This LED serves two functions: a. **Constant red**: Indicates that the pump has shut down due to a mechanical or electrical problem. This includes a shutdown due to a high or low pressure limit being exceeded. Pressure limits are set in the Manual and Run screens. b. **Flashing red**: Occurs during a method run and indicates that a pre-programmed ALARM step has been reached. The system operator must respond before the method continues. Also, the Controller emits an audible tone. c. **Constant green**: Indicates the pump is running.

Table 2-5. (continued)
Workstation Front Panel Controls

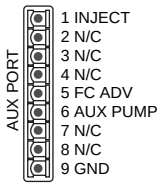
Feature	Description
Plumbing Connections	**Ports on the front of the Workstation:** a. **Pumphead Inlet ports:** Buffer inlet lines attach to the bottom of each pumphead using standard 1/4-28 flat-bottom fittings. The pumphead inlet tubing is 1/8” (3.2 mm) OD, 0.062” (1.6 mm) ID PTFE tubing with flat bottom fittings which are supplied in the Fittings kit. b. **Pumphead Outlet and Pressure Transducer Inlet and Outlet ports:** All plumbing following the pumphead outlet ports uses standard 1/4-28 flat-bottom fittings and the following tubing: **Orange PEEK tubing**: 1/16” (1.6 mm) OD, 0.020” (0.51 mm) ID. Used with pressures up to 5000 psi. **Green PEEK tubing**: 1/16” (1.6 mm) OD, 0.030” (0.76 mm) ID. Used with pressures up to 3000 psi, usually for flow rates greater than 20 ml/min. c. **Pumphead Priming ports:** This port on each pumphead is used to prime the pump. The port accepts any size syringe with a luer fitting; a syringe is included in the Fittings kit. This draws buffer through the inlet line and to the pumphead. Twist the port counter-clockwise one turn to open it. d. **Pumphead Washout Inlet ports:** The port on top of each pumphead is used to rinse the piston to remove crystallized salts. It accepts any syringe with a luer fitting. A syringe for this purpose is included in the Fittings kit. The rinse output is the open trough between the pumpheads. The pumpheads should br rinsed daily.

Table 2-6.
Workstation Rear Panel Connectors



Connector	Description
	Solenoid Valves: To connect DuoFlow low pressure solenoid valves (SV5-4 and SVT3-2) to the system. If a Maximizer is in use, connect to its solenoid valve connectors before those on the Workstation.
	Automated Valves: To connect DuoFlow high pressure automated valves (AVR7-3 Inject and AVR9-8 Stream Select) to the system. If a Maximizer is in use, connect to its automated valve connectors before those on the Workstation.
	Cond Flowcell: To connect the Conductivity monitor flow cell to the system. If the Maximizer is being used, you must use its Cond Flowcell connector rather than the connector on the Workstation.
	UV Lamp: This specialized 6-pin square connector provides electrical power to the mercury or zinc lamp in the UV detector lamp housing. This connector is not available on the Maximizer.
	UV Optics: To connect the UV detector to the system.
	Mixer: To connect the mixer to the system. If the Maximizer is in use, connect to its mixer connector before those on the Workstation.
	10...25V --0.3A Max: Provides electrical power to the Model 1327 chart recorder or Instrument Control Module (ICM).
	Cond Chart: For conductivity signal output to a single or dual pen chart recorder. An 8-pin mini-DIN to banana plug cable (**System Cable 4**) for connection to the Model 1327 chart recorder is available. Connect the red line to the positive (+) terminal and the black line to the negative (–) or ground terminal of channel 2 (CH2). The chart recorder should be set to 1 V full scale. If you are using the Model 1327 chart recorder, move all switches to the “green” settings.

Table 2-6. (continued)
Workstation Rear Panel Connectors

Conector	Description
	**UV Chart**: For UV signal output to a single or dual pen chart recorder. When the Bio-Rad Model 1327 is used, chart recorder Pen Up/Down, Stop/Start commands, and event marks are sent from this connector. The Bio-Rad Model 1327 dual pen recorder needs an 8-pin mini-DIN to standard DIN cable (System Cable 2) and a mini-DIN to banana plugs cable (System Cable 4). Generic chart recorders require an 8-pin mini-DIN to breakout cable (System Cable 7), available from Bio-Rad. When a Signal Import Module signal replaces the standard BioLogic UV Detector, use System Cable 20 to control a Bio-Rad Model 1327 dual pen chart recorder. The chart recorder should be set to 1V.
	**Power Cord:** The grounded 3-prong connector inputs power to the Workstation and outputs power to any unit connected to the Workstation. The Workstation's input power cord should be plugged into a 3-prong grounded power outlet.
	**Instr Bus:** The RJ-45 modular phone connectors and the bus communication cables connect the Workstation to the other components in the system. The Instrument Bus handles all communications between the Controller and each of the components in the system. For example, the Instrument Bus connects the Workstation to the USB Bitbus Communicator, Maximizer, BioFrac fraction collector, or Econo Gradient Pump. Components can be connected to the system in any order.
	**Aux:** The 9-pin AUX PORT connects a variety of peripheral modules that cannot communicate with the DuoFlow Controller over the Instrument Bus. If the Maximizer is being used, use its AUX connector before the connector on the Workstation. **Pin # Description** 1 **Inject.** A contact closure between pins 1 and 9 (GND) satisfies a Hold command which has been programmed in a method protocol. 2 **n/c**. No connection 3 **n/c**. No connection 4 **n/c**. No connection 5 **FC Adv.** Model 2110 and generic fraction collector Advance output. 6 **AUX Pump.** A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1). 7 **n/c**. No connection 8 **n/c**. No connection 9 **GND**. Ground
	Reserved for internal Bio-Rad use.

2.3 BIOLOGIC MAXIMIZER VALVE SYSTEM

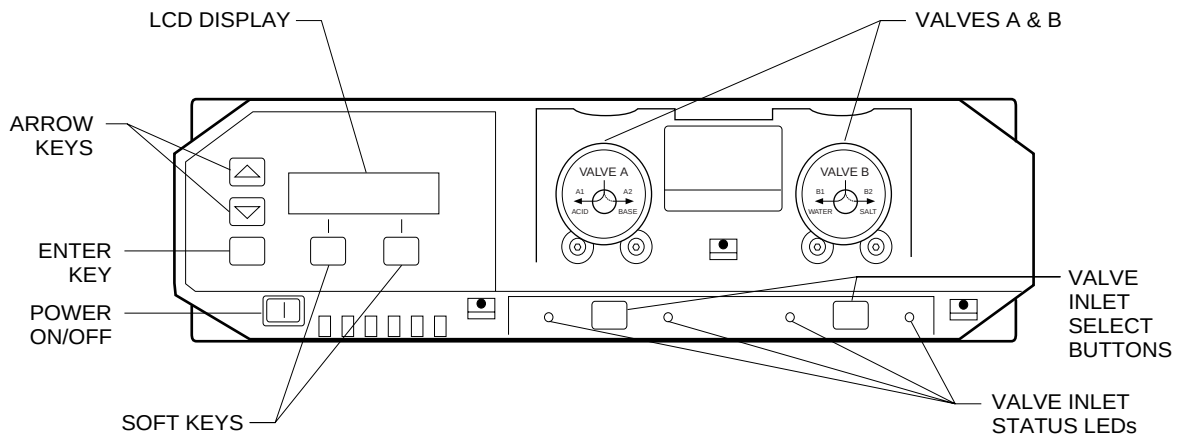
The Maximizer enables buffer blending applications, doubles the accessible pump flow rate, and doubles valving capacity to 6 low pressure valves and 6 high pressure valves. The Maximizer includes a separate Maximizer mixer (see Section 2.4.2) and pH monitor (see Section 2.5.4).

- Proportioning valves on the Maximizer blend water, salt, and the conjugate acid and base of a buffer to obtain a solution with a user-defined pH and salt concentration. One valve delivers an acid and base and the other valve delivers a salt and water.
- Pre-defined Buffer Blending buffer systems are provided for virtually all common buffers used in chromatographic applications. Additional user-defined buffers may be created using the BioLogic software Buffer Editor feature. The Maximizer uses the buffer system information to determine the amount of acid, base, water and salt to add to obtained the desired buffer composition and pH.
- When the Maximizer is set to local mode in the software Manual screen, it will not be under DuoFlow system control. In local mode the Maximizer front panel controls are accessible and can be used to prime the system, calibrate the pH and conductivity monitor, observe the status of each device, and control the position of each Maximizer valve.
- The Maximizer is designed to operate under normal laboratory and coldroom conditions (4° - 40° C) with all commonly used aqueous chromatographic buffers.

The Maximizer includes the following hardware and circuitry:

- Front panel: on/off switches; two 3-port, two position proportioning valves for automated buffer blending; an LCD screen and membrane switches for controlling valve positions and calibrating the pH probe and conductivity monitor.
- Rear panel: connectors that (with the exception of the UV detector) duplicate the connectors on the Workstation. It is recommended that valves, peripheral devices, and flow cells be connected to the Maximizer rather than the Workstation unless more valving capability is needed.

Table 2-7.
Maximizer Front Panel Controls

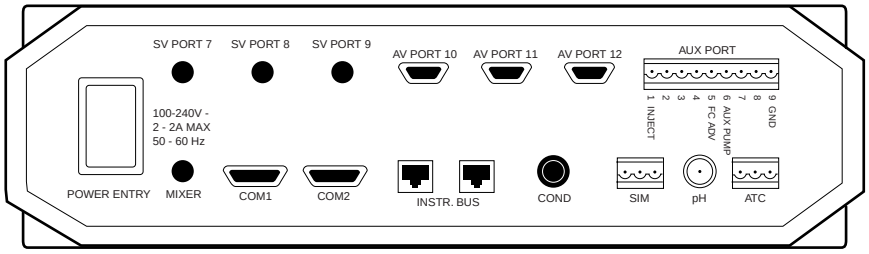


Feature	Description
Power On/Off	Controls power to the unit.
Valves A & B	Proportioning valves used for Buffer Blending and inlet selection. Each valve has two inlet ports and one outlet port. For Buffer Blending, acid is placed at inlet A1, base at inlet A2, water at inlet B1 and salt and inlet B2. The valve outlet ports are connected directly to their respective Workstation pump inlet ports. Color-coded tubing is supplied with the Maximizer for plumbing the inlet ports (A1 = red, A2 = blue, B1 = yellow and B2 = green).
Valve Inlet Select Buttons	Used when the Maximizer is in Local mode to change the position of valves A and B.
Valve Inlet Status LEDs	Indicates which valve port is open.
LCD Display	Displays the Maximizer status. When the Maximizer is operated from its front panel controls, the LCD displays status and control information, including valve positions, pH and conductivity calibration information, and current temperature.

Table 2-7. (continued)
Maximizer Front Panel Controls

Feature	Description
Softkeys	For limited local operation of the Maximizer. This is discussed further in Table 2-9.
Enter and Arrow Keys	To operate the Maximizer in conjunction with the softkeys, as discussed above.

Table 2-8.
Maximizer Rear Panel Connectors



MAXIMIZER REAR

Connector	Description
	Solenoid Valves: To connect DuoFlow low pressure solenoid valves (SV5-4 and SVT3-2) to the system. If a Maximizer is in use, connect to its solenoid valve connectors before those on the Workstation.
	Automated Valves: To connect DuoFlow high pressure automated valves (AVR7-3 Inject and AVR9-8 Stream Select) to the system. If a Maximizer is in use, connect to its automated valve connectors before those on the Workstation.
ATC	**ATC:** Reserved for future use.
	pH monitor: To connect the DuoFlow pH electrode. The pH monitor is described in greater detail in Section 2.5.4.
SIM	**SIM device:** The Signal Import Module (SIM), enables connection of a detector or device that outputs an analog signal between -2.5 Volts to +2.5 Volts. Instruments that may be connected in this way could include a variable wavelength UV detector, a refractive index detector, or a fluorescence detector. The SIM digitizes the analog signal and transmits it to the BioLogic DuoFlow Controller. External SIM modules are discussed later in this chapter.
	Cond Flow cell: To connect the Conductivity flow cell. The Conductivity monitor flow cell must be connected to the Maximizer, to supply temperature information for pH compensation.

Table 2-8. (continued)
Maximizer Rear Panel Connectors

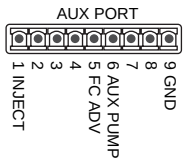
Connector	Description
	**Instrument Bus:** The RJ-45 modular phone connectors and their bus communication cables connect the Maximizer to the Controller and the Workstation (via the USB Bitbus Communicator). The Instrument Bus handles all communications between the Controller and each of the components in the system. Components can be connected in any order in the system.
	**Com 1:** To connect the QuadTec UV/Vis detector.
	**Com 2:** Reserved for future use.
	**Mixer:** To connect the mixer.
	**Aux:** The 9-pin AUX port connects a variety of peripheral modules that cannot communicate with the DuoFlow Controller over the instrument bus. **Pin # Description** 1 **Inject.** A contact closure between pins 1 and 9 (GND) satisfies a Hold for inject command which has been programmed in a method protocol. 2 **n/c**. No connection 3 **n/c**. No connection 4 **n/c**. No connection 5 **FC Adv.** Model 2110 and generic fraction collector Advance output. 6 **AUX Pump.** A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1 pump). 7 **n/c**. No connection 8 **n/c**. No connection 9 **GND**. Ground
	**Power Cord:** The grounded 3-prong connector inputs power to the Workstation and outputs power to any unit connected to the Workstation. The Workstation's input power cord should be plugged into a 3-prong grounded power outlet.

Table 2-9.
Maximizer Screens

Screen	Function and Description on Maximizer Faceplate in Local Mode
BLEND A POS 0 PREV NEXT	**Inlet Selection** Arrow buttons switch between Inlets A1(0) and A2(1). PREV changes to previous screen. NEXT changes to next screen.
BLEND B POS 1 PREV NEXT	Arrow buttons switch between Inlets B1(0) and B2(1).
<Valve><Port> POS 0 PREV NEXT	**Valve Control** Arrow buttons select a port <Port> on valve <Valve>. Up to six valves may be displayed including three motorized (AVR7-3, AVR9-8) and three solenoid (SVT3-2, SV5-4) valves. ENTER accepts the change and moves the valve to the new position.
SIM 0.000 Volts PREV NEXT	**SIM Calibration** Displays the current Maximizer SIM voltage.
CAL SIM? ENT=SET PREV NEXT	Used in conjunction with a 1 Volt calibration source. Pressing ENTER sets the current voltage reading to 1 V. Pressing the UP arrow resets the calibration to the factory setting.
CAL SIM? ENT=SET PREV set NEXT	After ENTER is pressed, the display responds with “SET” to show that the calibration was successful.
CAL SIM? ENT=SET PREV reset NEXT	After the UP arrow is pressed, the display responds with “RESET” to show that the calibration was reset.
pH 6.00 22.6 C PREV NEXT	**pH Calibration** Displays the current pH and temperature.
CAL pH? ENTER = Y PREV NEXT	ENTER causes the Maximizer to enter pH calibration mode.

Table 2-9. (continued)
Maximizer Screens

Screen	Function and Description
	pH Calibration (continued)
1ST pH PT. _4.00 CURSOR CANCEL	CURSOR moves the cursor to the next digit of the pH set point. UP/DOWN arrows adjust the pH set point. CANCEL aborts the calibration. ENTER accepts the pH value of the first calibration buffer and moves to the next screen.
1ST: 35.22 mV CANCEL SET	Display of the current pH voltage for monitoring probe equilibration. SET or ENTER sets the first calibration point. CANCEL aborts the calibration.
2ND pH PT. _7.00 CURSOR CANCEL	CURSOR moves the cursor to the next digit of the pH set point. UP/DOWN arrows adjust the pH set point. CANCEL aborts the calibration. ENTER accepts the pH value of the second calibration buffer and moves to the next screen.
2ND: 35.22 mV CANCEL SET	Displays the current pH voltage for monitoring probe equilibration. SET or ENTER sets the second calibration point and exits pH calibration mode. CANCEL aborts the calibration.
	Conductivity Calibration
COND. 0.86 PREV NEXT	Displays the current conductivity reading (mS/cm).
SET CELL C? ENT=Y PREV NEXT	ENTER causes the Maximizer to enter calibration mode.
NEW CELL C: 43.00 CURSOR CANCEL	CURSOR moves the cursor to the next digit of the calibration constant. ENTER accepts the conductivity cell constant, calibrates the conductivity meter, and exits calibration mode. CANCEL aborts the calibration.
	Beeper
BEEPER? ENTER=Y PREV NEXT	ENTER causes the Maximizer to enter volume adjustment mode.
VOLUME: UP/DOWN ENTER TO FINISH	The UP and DOWN arrows adjust the volume of the alarm.
	Firmware Version
MAXIMIZER V3.xx NEXT	Displays the current version of the Maximizer firmware.

2.4 MIXERS

Bio-Rad's DuoFlow mixers improve gradient quality by mixing the output from the DuoFlow Workstation pumps. There are two PEEK biocompatible mixers for DuoFlow systems.

- MX-1 mixer: A low volume mixer for use with a DuoFlow system ***not*** equipped with the Maximizer.
- Maximizer mixer: A large volume mixer for use with the Maximizer.

The mixer cable plugs into the connector labeled **Mixer** on the rear of the Maximizer or Workstation. If you will be using the MX-1 mixer, connect it to the Workstation; if you will be using the Maximizer mixer, connect it to the Maximizer. The mixers have two inlet ports; seal the unused port with the plug provided.

2.4.1 MX-1 Mixer

The Model MX-1 mixer is used when the DuoFlow system is **not** equipped with the Maximizer. The mixer may be used with or without a mixer barrel: the mixer body, mixer top, and mixer barrel are provided with the system; an optional mixer barrel extender is available. The mixer barrels fit between the mixer body and the mixer top and are used for higher flow rates. Refer to the following table to determine the appropriate mixer volume.

Table 2-10.
Mixer Barrels and Mixer Capacity for the MX-1 Mixer

Flow Rate	Barrel Extension	Capacity	Assembly Screws
less than 1ml/min	none	263 µl	10-32 x 5/8" (1.6 cm)
1 to 10 ml/min	750 µl Barrel Extender	750 µl	10-32 x 7/8" (2.2 cm)
10 to 40 ml/min	2.0 ml Barrel Extender	2.0 ml	10-32 x 1-1/2" (3.8 cm)

The procedure for changing the mixer capacity is provided at the end of this section.

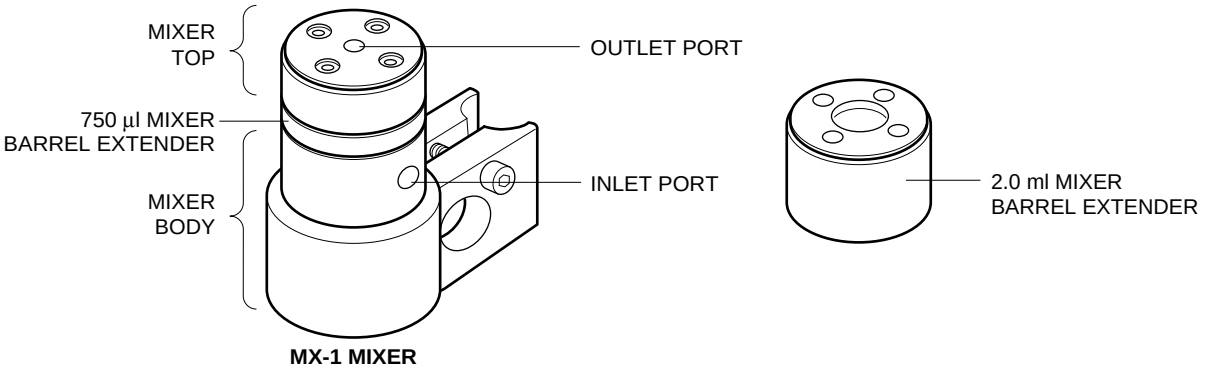


Figure 2-2. MX-1 Mixer and Mixer Barrel Extender

2.4.2 Maximizer Mixer

The Maximizer mixer provides the larger capacity required when the Maximizer is used. The Maximizer mixer may be used with or without a mixer barrel: the mixer body, mixer top, and mixer barrel are provided with the system; an optional mixer barrel extender is available. The mixer barrels fit between the mixer body and the mixer top and are used for higher flow rates. Refer to the following table to determine the appropriate mixer volume.

Table 2-11.
Mixer Barrels and Mixer Capacity for the Maximizer Mixer

Flow Rate	Barrel Extension	Capacity	Assembly Screws
0.5 to 10 ml/min	none	750 µl	1/4-20 x 1/2" (1.3 cm)
10 to 40 ml/min	5 ml Barrel Extender	5 ml	1/4-20 x 1 1/2" (3.8 cm)
40 to 80 ml/min	12 ml Barrel Extender	12 ml	1/4-20 x 3 1/4" (8.2 cm)

The procedure for changing the mixer capacity is provided in the following section.

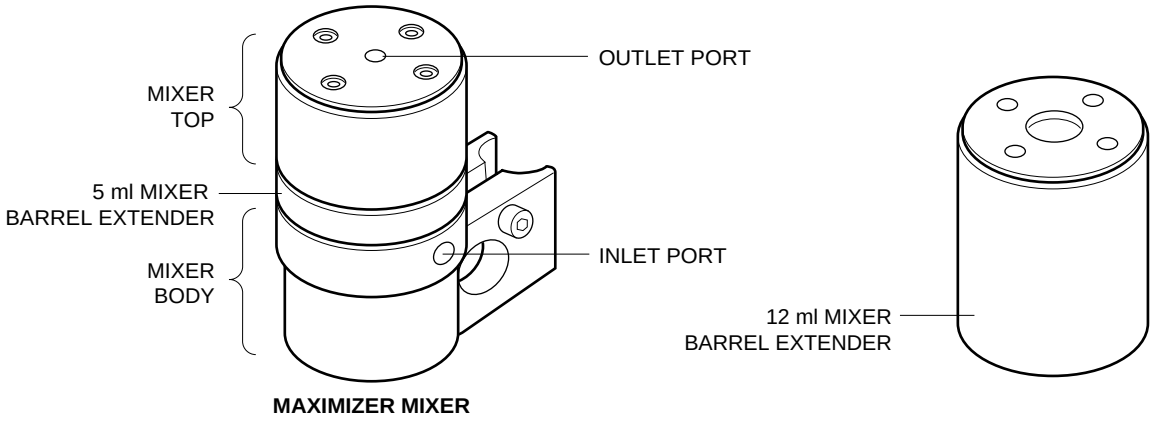


Figure 2-3. Maximizer Mixer and Mixer Barrel Extender

2.4.3 Changing Mixer Capacity

Be sure to follow these directions carefully. The mixer may leak if it is assembled incorrectly, or if an O-ring is not correctly placed in the O-ring groove.

Note: Flush any hazardous material from the system. Drain fluid from the mixer and disconnect the mixer plumbing and cables.

1. If you will be changing the mixer capacity, use the information in Table 2-10 and Table 2-11 to select the appropriate screws and barrel extension.
2. Use the hex key provided to remove the four screws from the top of the mixer (see Figure 2-4).
3. Remove the mixer top and turn it upside down to remove the O-ring. If the O-ring does not easily dislodge, use your fingers to remove it.
4. Refer to Figure 2-4 for re-assembling the mixer. Make sure that the magnetic stir bar lies flat. If the standard mixer barrel is to be used, assure the O-ring groove faces up.
5. Place an O-ring in each O-ring groove. If you are using only the mixer body, only one O-ring is required. If the mixer barrel is used, two O-rings are required.

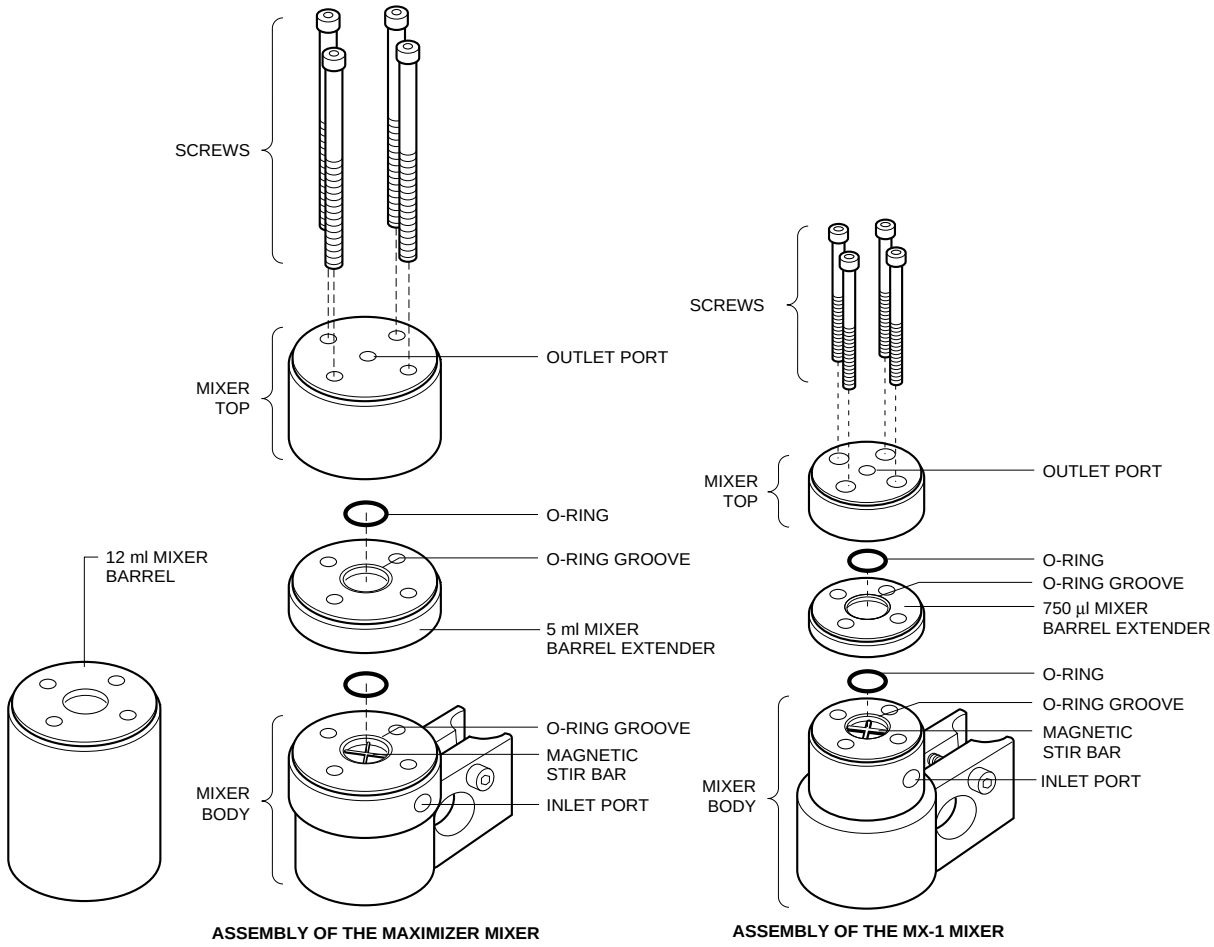


Figure 2-4. Assembly of Mixers

2.5 DETECTION SYSTEMS

The DuoFlow system supports the following detection and monitoring devices:

- UV Detector
- Conductivity Monitor
- QuadTec UV/VIS Detector
- pH Monitor

2.5.1 UV Detector

The UV detector is a single beam, fixed wavelength UV absorbance detector specifically designed for high resolution protein chromatography.

- Available in several configurations.
- Rack mountable and portable, which enables it to be positioned close to a column outlet for better resolution and decreased peak band broadening.
- Optional 214 nm filter and zinc lamp available for sensitive peptide analysis.

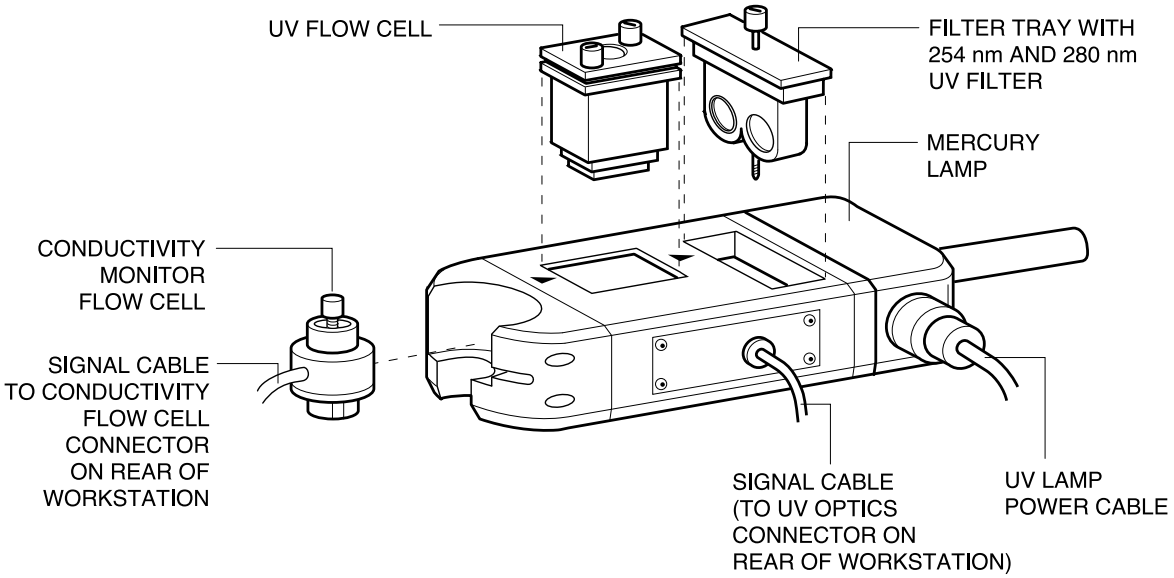


Figure 2-5. UV Detector, with Mercury Lamp, 254 & 280 nm Filters, and Conductivity Flow Cell

The UV detector consists of the optics bench, a filter tray, a flow cell, and a lamp. It is designed to hold the Conductivity monitor flow cell. The following configurations are available:

- **Mercury Lamp and Filters.** The mercury lamp comes installed in the optics module along with 280 nm and 254 nm filters. The filters are both held by a single tray. To switch filters, rotate the filter holder. Filters for different wavelengths are also available from Bio-Rad. These include 365 nm, 405 nm, 436 nm, and 546 nm filters.
- **Zinc Lamp and 214 nm Filter.** The zinc lamp and 214 nm filter are optional. The zinc lamp attaches directly to the optics module in place of the mercury lamp. (Refer to Figure 2-6)

Two flow cells are available with the detector. Both use 1/4-28 flat-bottom fittings.

- Analytical 5 mm flow cell. This flow cell is recommended for high resolution detection. This flow cell has a 5 mm path length, a volume of 16 µl, and is rated to 750 psi at flow rates between 0.1 and 10 ml/min.
  To reduce the risk of an entrapped air bubble causing an unstable baseline, the 5 mm flow cell should be used with the backpressure regulator (see page 2-52). Connect the backpressure regulator after the Conductivity monitor in the system plumbing.
- Preparative 2 mm flow cell. This flow cell is recommended for most applications which demand less sensitivity, for flow rates greater than 10 ml/min, or when working with high protein concentrations. It has a 2 mm path length, a volume of 30 µl, and is rated to 750 psi.

The UV detector receives power via the Workstation, to which it is connected by the UV lamp cable. The UV detector communicates with the system via the UV optics cable, which plugs into the UV optics connector on the back of the Workstation.

The UV detector sensitivity ranges from 0 to 2.0 AUFS (Absorbance Units Full Scale). The UV sensitivity range for the chart recorder is set in the system software in either the Manual or Run screens. A Zero Baseline button is available in the Manual and Run screens and a programmable Zero Baseline command is available as part of a method protocol. Refer to the discussion of the chart recorder for setting the chart recorder range, page 2-55.

To replace an expired lamp, refer to Chapter 11, Maintenance, or to the instruction sheet for the replacement lamp.

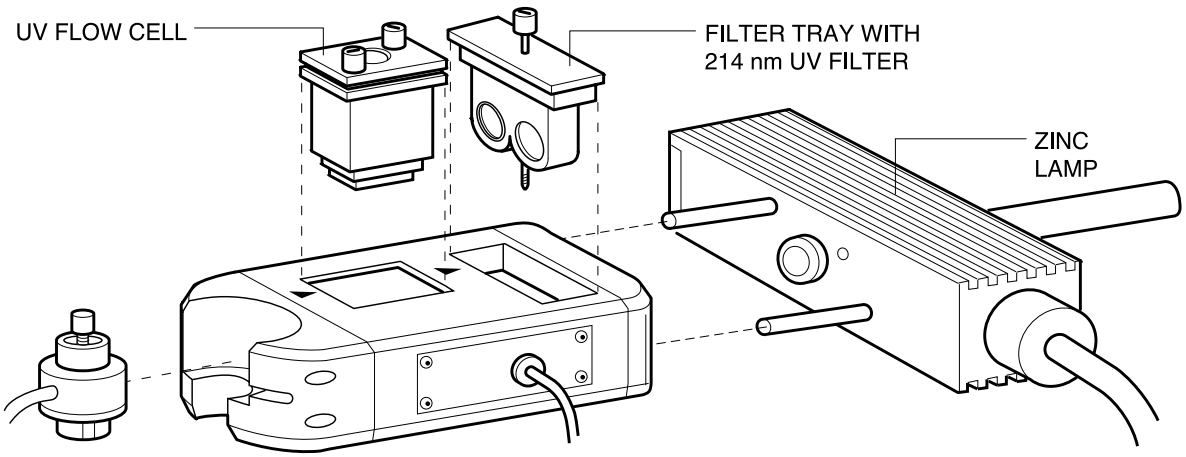


Figure 2-6. UV Detector, with Zinc Lamp, 214 nm Filter, and Conductivity Flow Cell

To change the UV flow cell or UV filter:

If the system has been used, make sure that any hazardous material has been flushed from the system and the pumps are not running. Drain fluid from the UV detector and disconnect its plumbing and cables.

1. To remove the UV flow cell, loosen the flow cell thumbscrews and lift the flow cell out.
2. To insert a flow cell, place it into the UV detector and tighten the thumbscrews.
3. To change the UV filter, loosen the flow cell thumbscrews and lift the filter tray out.
4. Rotate the filter tray to use the correct filter, and then place it into the UV detector and tighten the thumbscrews.

2.5.2 Conductivity Monitor

The Conductivity monitor, included with all DuoFlow systems, measures fluid conductivity to track the accuracy of a salt gradient. This data is useful for optimizing purification protocols and column cleaning procedures. Conductivity monitor sensitivity ranges from 0 to 500 mS/cm. The Conductivity monitor consists of the following:

- Flow cell with Inlet/Outlet ports: To connect the tubing, use 1/4-28 flat bottom fittings. The flow cell allows flow in either direction. The flow cell can be plumbed immediately after the UV flow cell or at any other point in the flowpath. It is designed to fit into a receptacle on the UV detector (see Figure 2-7). The volume in the cell is a nominal 6 µl.
- Signal cable: To connect to the back of the Workstation. Electrical power for the Conductivity monitor is drawn from the signal cable. If a Maximizer is in use, connect to its COND port.

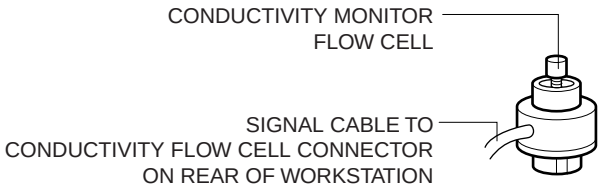


Figure 2-7. Conductivity Monitor

The Conductivity monitor can be calibrated by either entering the cell constant or by calibrating the cell against a known standard. The **Conductivity Flow Cell Constant Calibration** feature is found under the Utilities drop-down menu. The cell constant can be found on the tag attached to the cable.

The Conductivity sensitivity range for the chart recorder is set in the system software Manual and Run screens. When setting the range, choose one that will accommodate the maximum conductivity expected during the chromatography run. Keep in mind that although the chart recorder range can be manipulated during a chromatography run, the data will be stored unattenuated, allowing for re-scaling at a later time.

2.5.3 QuadTec UV/VIS Detector

The BioLogic QuadTec UV/Vis detector enables four wavelengths to be monitored simultaneously and displayed on the DuoFlow Controller. The QuadTec detector has a wavelength range of 190-370 nm using the deuterium lamp and 370-740 nm with the halogen lamp. The deuterium lamp is standard and is required for the detection of peptides, proteins, and nucleic acids. Both lamps are pre-aligned, calibrated, and user-serviceable.

Wavelengths are selected through the use of a moveable grating monochromator with an accuracy of +/- 1 nm in 1 nm steps. When using the deuterium lamp for the monitoring of wavelengths >380 nm, an automatic cutoff filter is activated. Because very little light of wavelengths greater than 400 nm is emitted from a deuterium lamp, the halogen lamp is required for routine detection above 380 nm.

The QuadTec includes the following:

- 3mm Peek Flow Cell (1 µl flow cell volume, 4 µl including flow cell inlet and outlet tubes)
- 10-32 Fingertight fittings (quantity 4)

- System Cable 25, RS232. To connect to the Instrument Control Module (ICM). If a Maximizer is in use, connect to its COM1 port instead of the ICM.
- Power cord

An optional 2 mm PEEK flow cell (2.2 µl flow cell volume, 18 µl including flow cell inlet and outlet tubes) is available for flow rates up to 80 ml/min including 10-32 long Fingertight fittings (quantity 4).

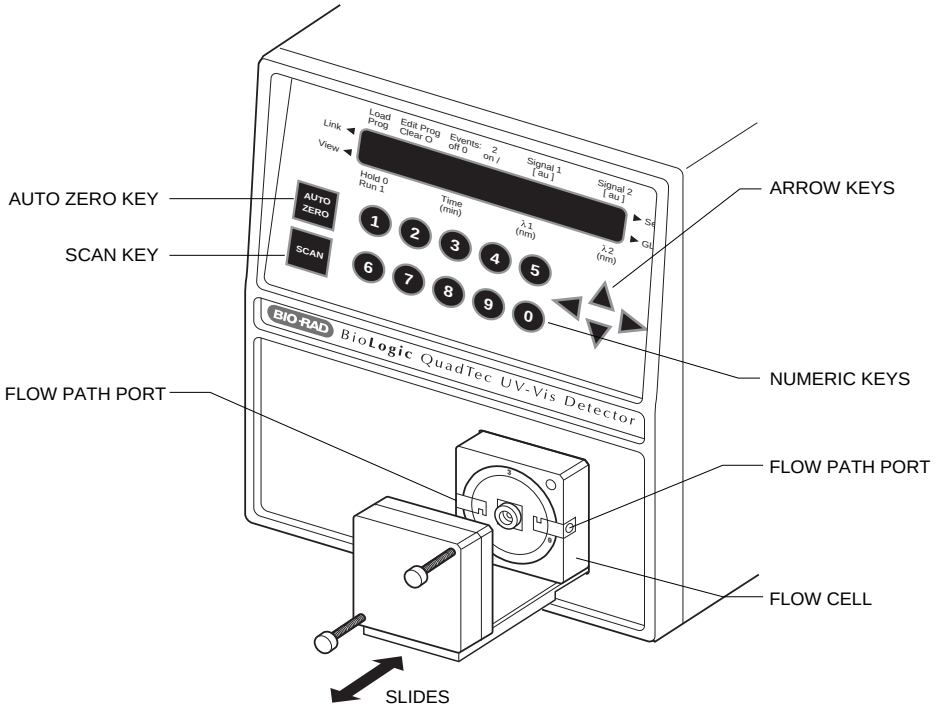


Figure 2-8. QuadTec UV/Vis Detector

For a complete discussion of the QuadTec detector, refer to its separate documentation.

2.5.4 pH Monitor

The BioLogic DuoFlow pH monitor enables direct monitoring of pH conditions during a run. The pH monitor is optional for all DuoFlow systems.

The pH monitor consists of the following:

- Flow cell: The PEEK flow cell has a swept volume of approximately 80 µl when the pH electrode is inserted. The flow cell mounts to the BioLogic rack using an attached mounting bracket, which can also be used to attach the UV detector and Conductivity monitor. The flow cell should be positioned so that the flow path is angled upward to promote bubble clearance. Plumb the flow cell with the inlet tube attached to the lower port and the outlet tube attached to the upper port. The inlet and outlet ports are threaded for use with 1/16” (1.6 mm) OD tubing and 1/4-28 flat bottom fittings. The pH monitor is designed for flow rates up to 80 ml/min and flow cell pressure less than 75 psi, and therefore should be plumbed downstream of the backpressure regulator.
- pH electrode: The pH probe is a sealed Calomel type electrode consisting of a pH and a reference electrode built into the same body. The sealed reference design eliminates the need to add electrolyte solutions and minimizes reference dryout. The Calomel electrode is fully compatible with buffers (such as Tris) that may not be compatible with the Ag/AgCl (silver/silver chloride) containing electrodes.
- Signal Cable: To connect the pH probe to the BNC connector of a Signal Import Module or Maximizer.

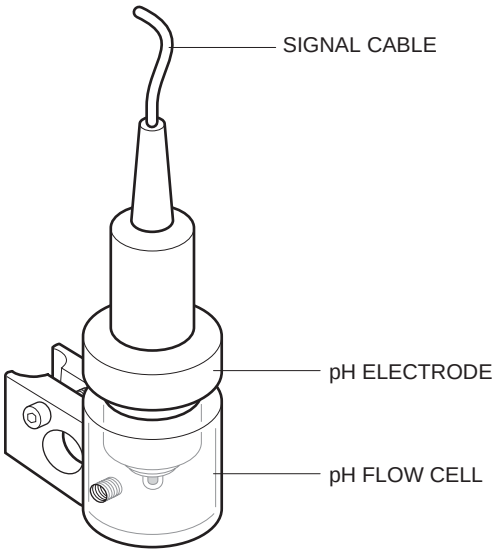


Figure 2-9. pH Monitor

The pH electrode and flow cell are described in detail in their separate documentation.

2.5.5 Other Detectors

Detectors other than those offered by Bio-Rad may be used when connected to the Signal Import Module (SIM.) Refer to Section 2.9.6.

2.6 VALVES

BioRad offers an extensive variety of valves that enable greater flexibility for multiple sample and advanced application options. The DuoFlow system controls 3 AVR high pressure valves and 3 SV low pressure valves. DuoFlow systems with a Maximizer double the number of valves to 6 AVR high pressure and 6 SV low pressure valves. Methods that include automated injection, buffer selection, column switching, and large volume fraction collection are easily performed using various valve configurations.

For further discussion of valving applications and protocols, refer to Chapters 8-9.

2.6.1 AVR7-3 Sample Inject Valve

The AVR7-3 sample inject valve is a 7-port, 3-position valve for injecting samples and is an essential component of all DuoFlow systems. It is rated to 3500 psi (233 bar) and is designed with non-metallic wetted parts and minimal internal dead volume. Some features of this valve include:

- Patented make-before-break design (MBB™) prevents pressure spikes when the valve rotates from one port to another, eliminating baseline interferences and prevents pump shutdowns due to transient over pressure situations. This is especially beneficial when using more fragile low pressure columns or flow sensitive detectors (e.g. refractive index).
- Applications include sample injection, reverse flow chromatography and two-column switching.
- A valve rebuild kit is available.

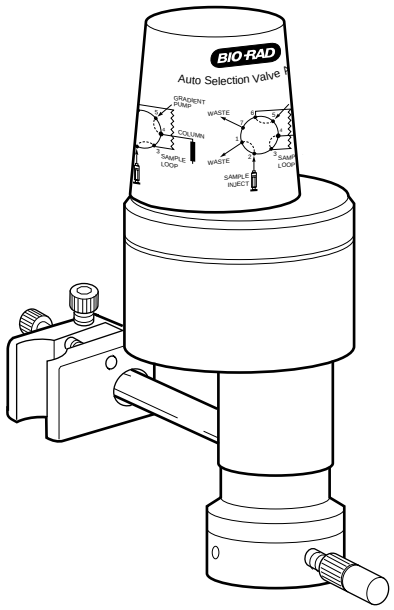


Figure 2-10. AVR7-3 Sample Inject Valve

The three valve positions are Load (position 1), Inject (position 2), and Purge (position 3). See Figure 2-11. Load is the default position when the system is powered up or at the end of a method run unless configured differently from the Edit User Preferences window, available from the Options menu in the system software.

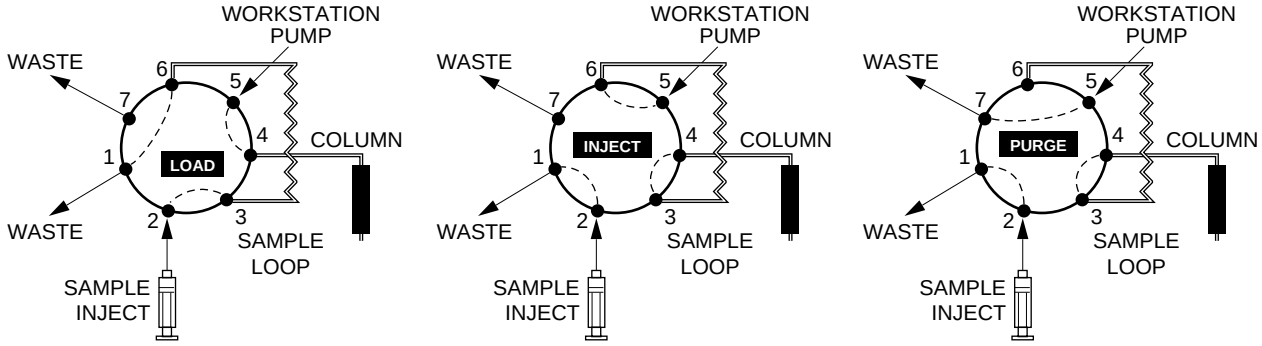
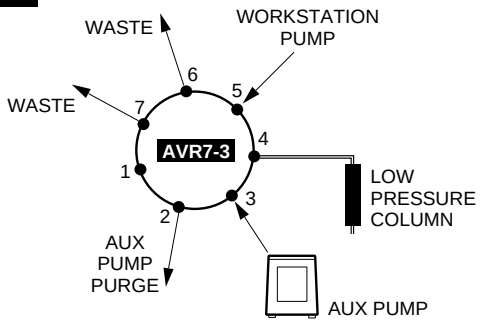


Figure 2-11. Sample Load Positions

The valve uses 1/16” (1.6 mm) OD tubing and 1/4-28 fittings. Sample loop sizes are available from 50 µl loop (in the Starter kit included with all systems) to a maximum of 5 ml PEEK loop. Larger volume injections may be obtained using Dynaloops, additional valves and/or an auxiliary pump.

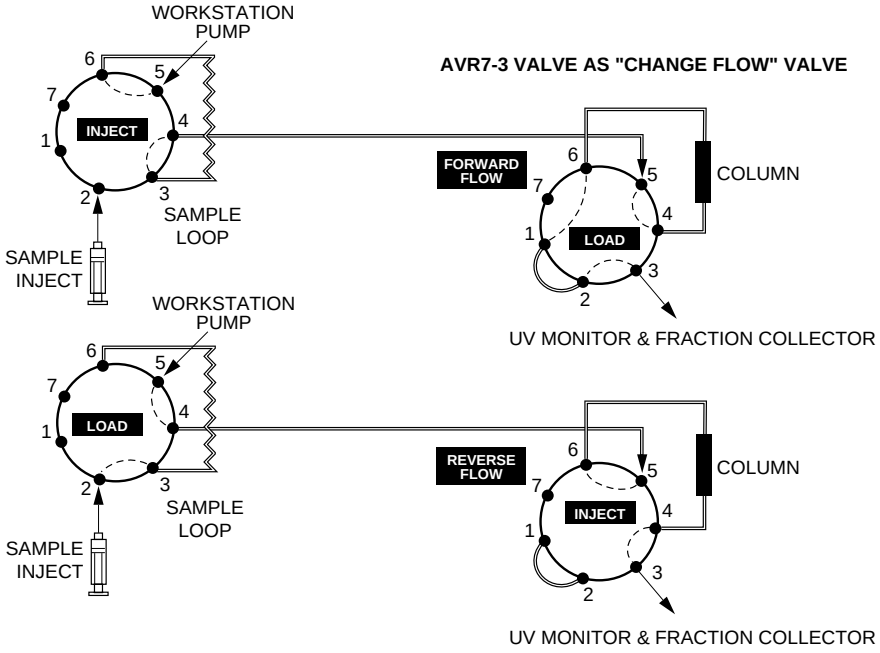
Connect the AVR7-3 valve signal cable to any of the available Automated Valve connectors on the back of the Workstation (ports 4, 5, or 6). If a Maximizer is in use connect to ports 7, 8, or 9 before those on the Workstation. If more than 3 valves are desired, you may connect additional. valves to ports 4, 5, and 6 on the Workstation. All valves will be active.

LARGE VOLUME SAMPLE LOADING



SAMPLE LOADING AND AFFINITY CHROMATOGRAPHY

AVR7-3 VALVE FOR SAMPLE LOADING



COLUMN SWITCHING

AVR7-3 AS COLUMN SWITCHING VALVE

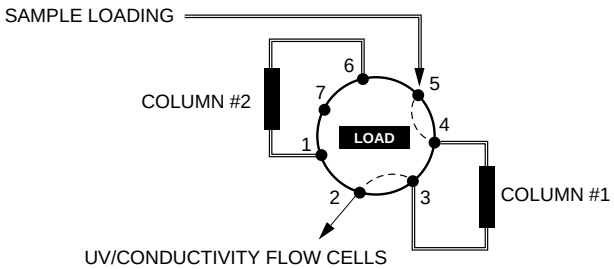


Figure 2-12. Examples of AVR7-3 Valve Tubing

2.6.2 AVR9-8 Stream Select Valve

The AVR9-8 stream select valve is an 9-port 8-position valve. This valve is optional for all DuoFlow systems. The valve is rated at 3500 psi (233 bar) and is designed with non-metallic wetted parts for bio-compatibility and minimal internal dead volumes.

- The AVR9-8 is ideal for stream selection, column switching, and large volume fraction collection.
- The AVR9-8 stream select valve uses a patented make-before-break design (MBB) that prevents pressure spikes when the valve rotates from one port to another, eliminating baseline interferences, and prevents pump shutdowns due to transient over pressure situations. This is especially beneficial when using more fragile low pressure columns or flow sensitive detectors (e.g. refractive index).
- A valve rebuild kit is available.

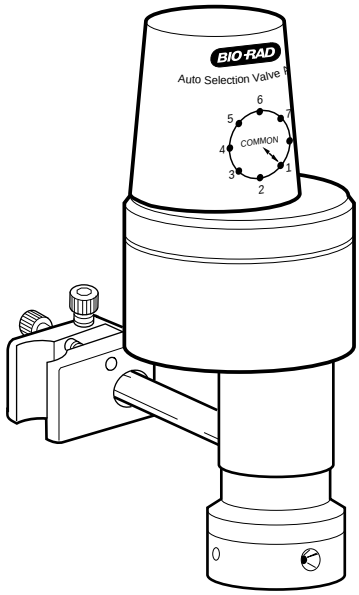


Figure 2-13. AVR9-8 Stream Select Valve

The valve's use dictates its plumbing. For example:

- When two AVR9-8 valves are used as a column switching valve, up to eight columns may be run sequentially.
- An AVR9-8 expands the number of available buffers or solvents when used as an Inlet Valve.
- When used in conjunction with an auxiliary peristaltic pump (such as the Bio-Rad Model EP-1 Econo pump or EGP Econo Gradient Pump) for loop filling and an AVR7-3 inject valve, the AVR9-8 valve may be used to select up to eight samples for consecutive chromatography runs.

Position 1 on the AVR9-8 is the default position when the system is powered up or at the end of a method run unless configured differently from the Edit User Preferences window, available from the Options menu.

The valve uses 1/4-28 fittings and 1/16” (1.6 mm) OD PEEK tubing supplied with the fitting kit when plumbing these valves.

Connect the AVR9-8 valve signal cable to any of the available automated valve connectors on the back of the Workstation (ports 4, 5, or 6). If a Maximizer is in use connect to ports 7, 8, or 9 before those on the Workstation. If more than three valves are desired, you may connect additional valves to ports 4, 5, and 6 on the Workstation. All valves will be active.

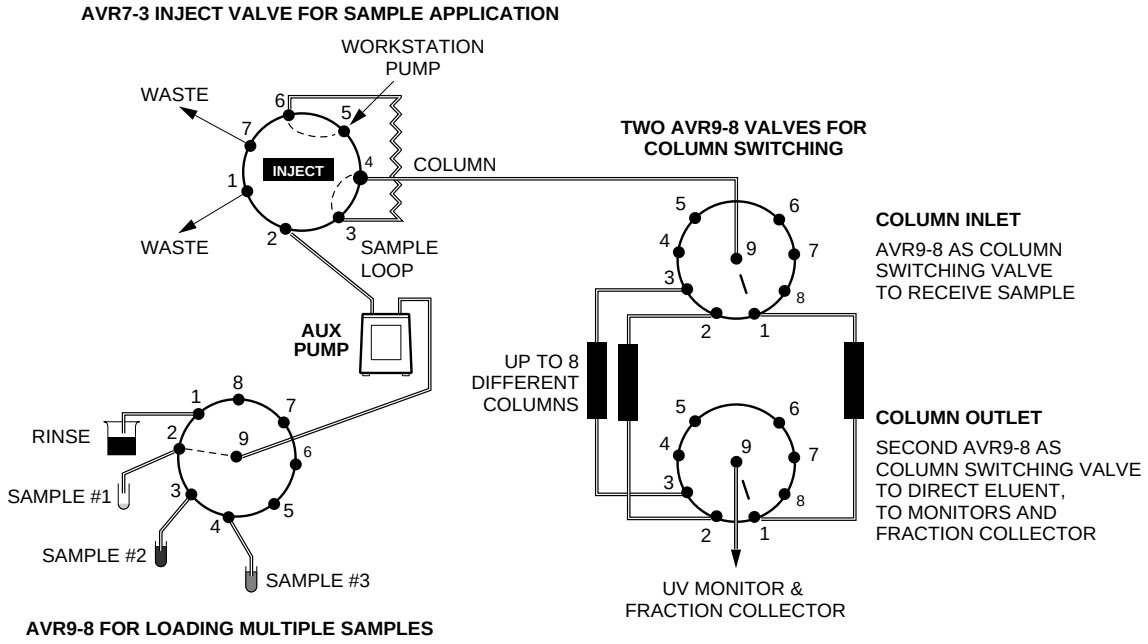


Figure 2-14. Two Examples Using AVR9-8 Valves

2.6.3 SV5-4 Buffer Select and Automated Sample Loading Valve

The SV5-4 valve is a low pressure, 5-port, 4-position valve used for automatic buffer selection and sample loading. The SV5-4 valve may be used for:

- Buffer and/or sample selection when placed before the Workstation pumps or an auxilary load pump. An SV5-4 valve connected to a Workstation pump inlet enables access to four separate solutions.
- Fraction collection when placed after the column.
- Purging or rinsing all tubing lines for cleaning purposes.

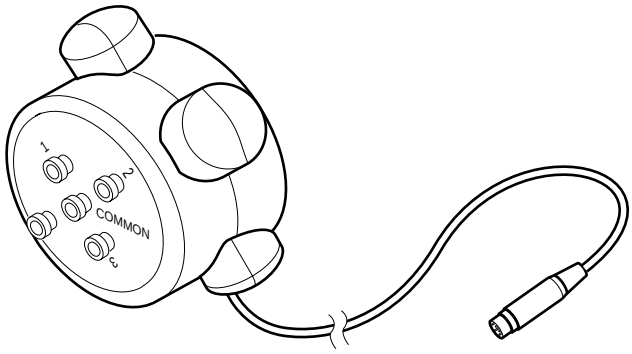


Figure 2-15. SV5-4 Buffer Select Valve

Use 1/8” (3.2 mm) OD PTFE tubing and 1/4-28 fittings when plumbing the SV5-4 valve to the Workstation pump inlet. To prevent air from entering the system, unused ports should be closed with 1/4-28 plugs.

> When loading samples directly through the Workstation pump, first filter the sample through a 0.45 µm filter. Flush the valve pump and lines with a sanitizing solution to remove protein residues after the sample has been loaded; protein residues might otherwise reduce pump piston seal lifetime.

Connect the SV5-4 valve cable to any of the available solenoid valve connectors on the back of the Workstation (ports 1, 2, or 3). If a Maximizer is in use, connect to ports 4, 5, or 6 before those on the Workstation.

From the Manual screen you may manually operate each inlet port on the SV5-4. Manual valve control is useful for the following:

- When priming the tubing and the valves with buffer and or sample prior to starting a method.
- For purging all tubing lines for cleaning purposes.

Prior to programming a method in the Protocol screen, you may name each of the valves positions in the Setup screen. The name that you apply in the Setup screen will appear in the method Protocol screen when you program an Isocratic Flow, Change Valve, or Linear Gradient step.

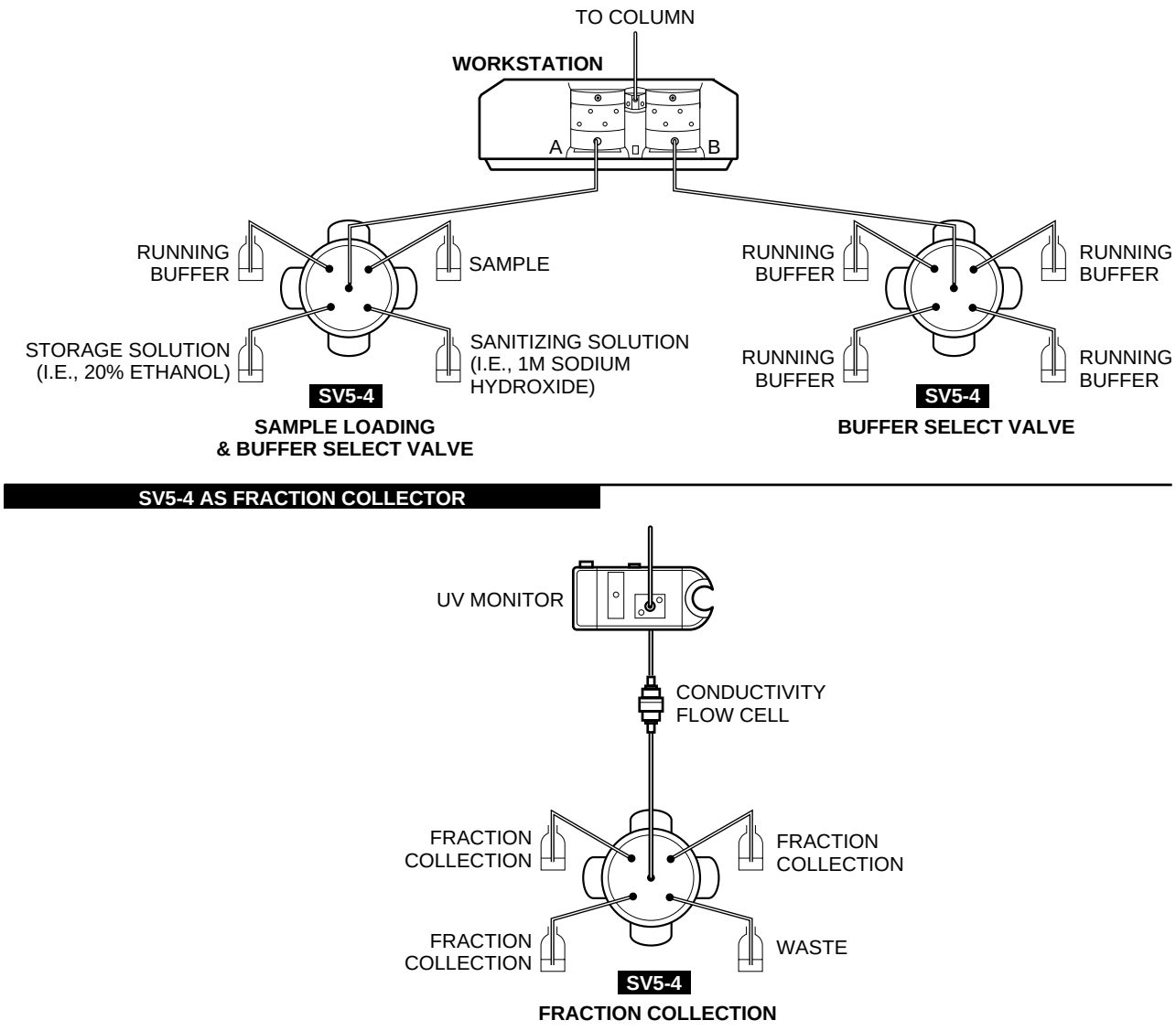


Figure 2-16. Two Examples using SV5-4 Valves

2.6.4 SVT3-2 Diverter Valve

The SVT3-2 diverter valve is a low pressure 3-port, 2-position valve. This valve may be used in a number of ways:

- To divert the eluant stream from the detectors to a fraction collector or a waste container.
- As a sample select valve when placed before Workstation pump A.
  The Maximizer may not be used in this application.
- As a water rinse valve when placed before Workstation pump B.
- As a user-define valve when included as part of the plumbing setup.

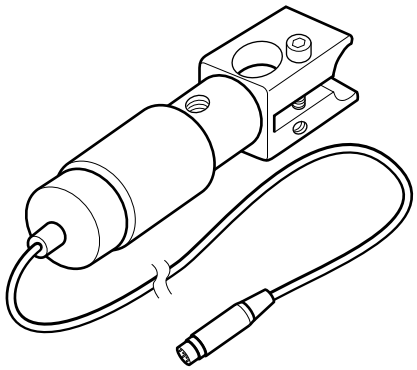


Figure 2-17. SVT3-2 Diverter Valve

Connect the SVT3-2 valve's signal cable to any of the available solenoid valve connectors on the back of the Workstation (ports 1, 2 or 3).

Plumbing connections depend on the valve's use:

- Plumbing as a diverter valve: Use 1/16" (1.6 mm) OD PEEK tubing and 1/4-28 fittings. Use orange PEEK 0.020" (0.51 mm) ID tubing for the F10 pumps, and green PEEK 0.030" (0.76 mm) ID tubing for the F40 pumps.
- Plumbing before a pump: Use 1/8" (3.2 mm) OD tubing and 1/4-28 fittings.

From the Manual screen you can manually switch inlet ports on the SVT3-2. Manual valve control is useful for the following:

- When priming the tubing and the valves with buffer prior to starting a method.
- For purging or rinsing all tubing lines during cleaning process.

In the Setup screen you can name each of the two valve positions. For example, when the SVT3-2 is placed before the Workstation pump, you can identify the valve by the name or composition of the buffer. The name that you apply in the Setup screen will appear in the method Protocol screen when you program an Isocratic Flow step, a Change Valve step, or a Linear Gradient step.

When used as a diverter valve with a Model 2110 or generic fraction collector, this valve should be named as such in the Setup screen. Position 1 of this valve is Waste; position 2, Collect.

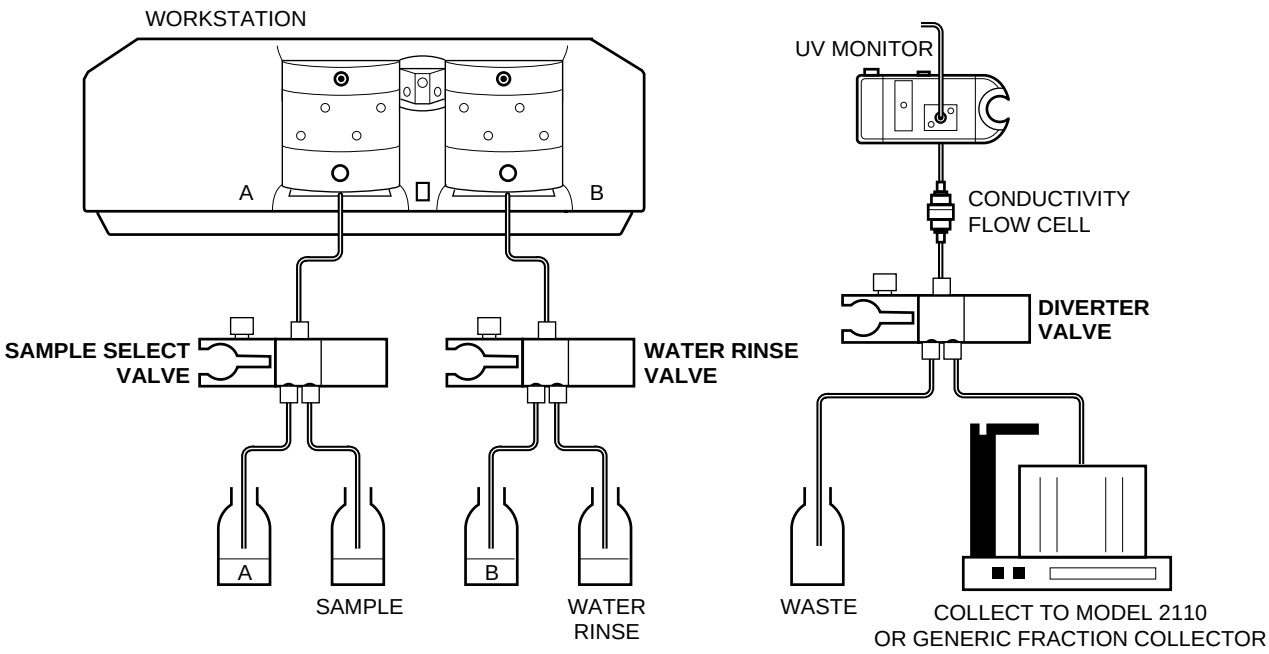


Figure 2-18. Two Configurations of the SVT3-2 Valve

When used as a diverter valve with a Model 2110 or generic fraction collector, this valve should be named as such from the Setup screen. Position 1 of this valve is Waste; position 2, Collect.

2.7 FRACTION COLLECTORS

The DuoFlow features several options for fraction collection:

- BioFrac fraction collector
- Model 2110 fraction collector
- Model 2128 fraction collector
- Non-Bio-rad fraction collectors

The BioFrac fraction collector, the Model 2110 fraction collector, and the Model 2128 fraction collector are programmable and can be run via BioLogic software version 4.0 or higher.

2.7.1 BioFrac Fraction Collector

The BioFrac functions with the BioLogic DuoFlow system and as a stand-alone fraction collector with the BioLogic HR and non Bio-Rad chromatography systems. It is suited for both analytical and preparative applications.

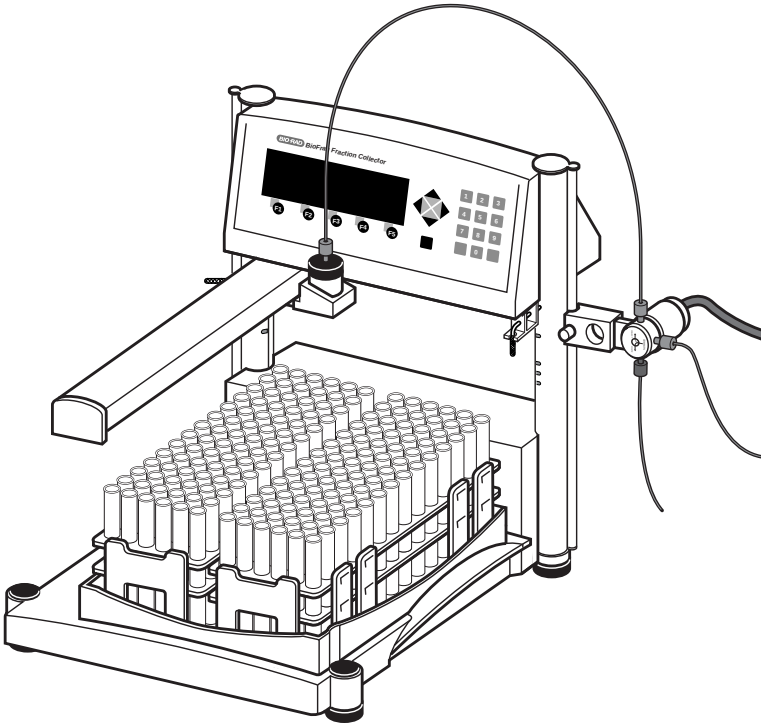


Figure 2-19. BioFrac Fraction Collector

Features of the BioFrac include:

- It can be used with any chromatography system at flow rates up to 100 ml/min.
- It can collect in time, drop or volume mode (time and drop mode are only available in stand-alone mode).
- A diverter valve, mounted on either side of the vertical bars, diverts unwanted eluant to waste.
- Can be fitted with an optional drop former (25 µl drops) designed for collecting small sample sizes.

- The BioFrac collects fractions in a serpentine pattern for all racks but may be changed to a row or column pattern for microplates and microtiter tubes. Fourteen collection choices are possible with a total of nine racks. The BioFrac accommodates 12-20 mm and 30 mm tube diameters, microtubes, and scintillation vials in a variety of diameters. The optional ice bath/microplate rack allows collection into 13 mm diameter tubes, microplates, and microtiter tubes that meet SBS standards required for automated microplate systems. The drop head height can be adjusted to accomodate tubes up to 150 mm.

The following racks are available.

Table 2-12.
BioFrac Racks Available

Rack Name	Description	Capacity (Format)
F1	12-13 mm diameter tube; 150 mm height	2 racks (6x15), 180 tubes
F2	15-16 mm diameter tube; 150 mm height	2 racks (5x12), 120 tubes
F3	18-20 mm diameter tube; 150 mm height	2 racks (4x10), 80 tubes
H1	1.5-2.0 ml capless microtubes	4 racks (6x7), 168 tubes
H2	0.5 ml capless microtubes	4 racks (7x9), 120 tubes
H3	16 mm scintillation vials	4 racks (5x6), 120 tubes
H4	30 mm scintillation vials	4 racks (2x3), 24 vials
H4-High	30 mm tubes	4 racks (2x3), 24 tubes
Ice Bath, Microplate rack	Ice bath for 13 mm diameter tubes, standard microplate or microtiter tubes (SBS standard format)	1 rack (10x12), 120 tubes 4 plates (12x8), 96 wells 4 plates (8x6), 48 wells 4 plates (4x6), 24 wells 4 plates (3x4) 12 wells
Prep-20	Preparative rack	bottle size fractions (20 funnels)

The BioFrac communicates with the DuoFlow Controller via the Instrument Bus. To connect the BioFrac to the bitbus use either of the instrument bus connectors on the rear of the fraction collector. The BioFrac fraction collector supports collection schemes such as Collect All, Threshold, Collection Windows, and Threshold + Collection Windows (all with Delay volume, if required). The BioFrac diverter valve can be mounted on either side of the fraction collector's upright supports. See Section 3.8.1 for more information on connecting the BioFrac to the DuoFlow system.

The BioFrac is plumbed to the DuoFlow system using 0.020" diameter PEEK tubing and 1/4-28 fittings for flow rates up to 20 ml/min or with 0.030" diameter PEEK tubing and 1/4-28 fittings for flow rates up to 100 ml/min.

This instrument is described in detail in its separate documentation.

2.7.2 Model 2110 Fraction Collector

The Model 2110 is programmed in the DuoFlow software and can function as a stand-alone fraction collector with non Bio-Rad systems. It uses a stationary, drop-dispensing head and collects up to 80 fractions in a motor-driven carousel. It also uses standard 13 x 100 test tubes. An optional adapter is available for use with 1.5 ml microcentrifuge test tubes. The Model 2110 includes the following features:

- In stand-alone mode the Model 2110 accepts small chromatography columns that can mount directly to the drop-former to minimize dead volume.
- It can collect from 1 drop (50 µl) to 9 ml fraction in the 13 x 100 test tubes or 1.5 ml micro test tubes.
- It is coldroom compatible.

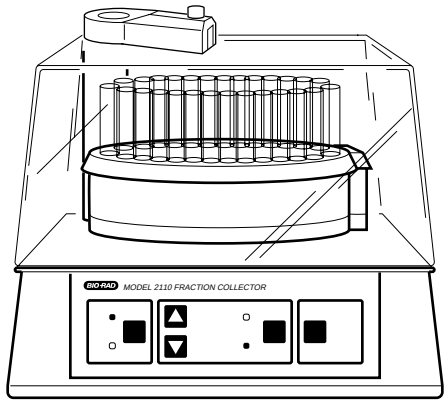


Figure 2-20. Model 2110 Fraction Collector with Optional Dust Cover

The Model 2110 is connected to the Maximizer or Workstation with system cable 5, bare wires to DB-9 via the AUX connector, black wire to pin 5 and white wire to pin 9. To prevent shorting, cut or tape wires not in use. If a Maximizer is being used, the Model 2110 should be connected to it rather than to the Workstation.

The Model 2110 is plumbed to the DuoFlow system using 1/16” (1.6 mm) OD inlet tubing connecting directly to the fraction collector’s drop former, without the need for additional fittings.

The SVT3-2 diverter valve is required by the Model 2110 for collection schemes other than “Collect All.” When this valve is configured as the *fraction collector diverter valve* in the Setup screen, additional collection parameters (Collect All, Threshold, Collection Windows, and Threshold + Collection Windows) become available in the Protocol screen.

When controlled by the DuoFlow system, fraction collection is specified by volume. The fraction collection scheme is programmed in the Protocol screen of the software where a Delay Volume feature is standard (see Chapter 7 for discussion of Delay Volume.)

Note: Collection by drop count is not available with the DuoFlow system.

This instrument is described in detail in its separate documentation.

2.7.3 Model 2128 Fraction Collector

The Model 2128 provides X/Y motion drop-dispensing across 5 available racks which accommodate a wide range of tube diameters and lengths, microtiter plates, microtubes, and “bottle size” fractions. It is suited for both analytical and preparative applications.

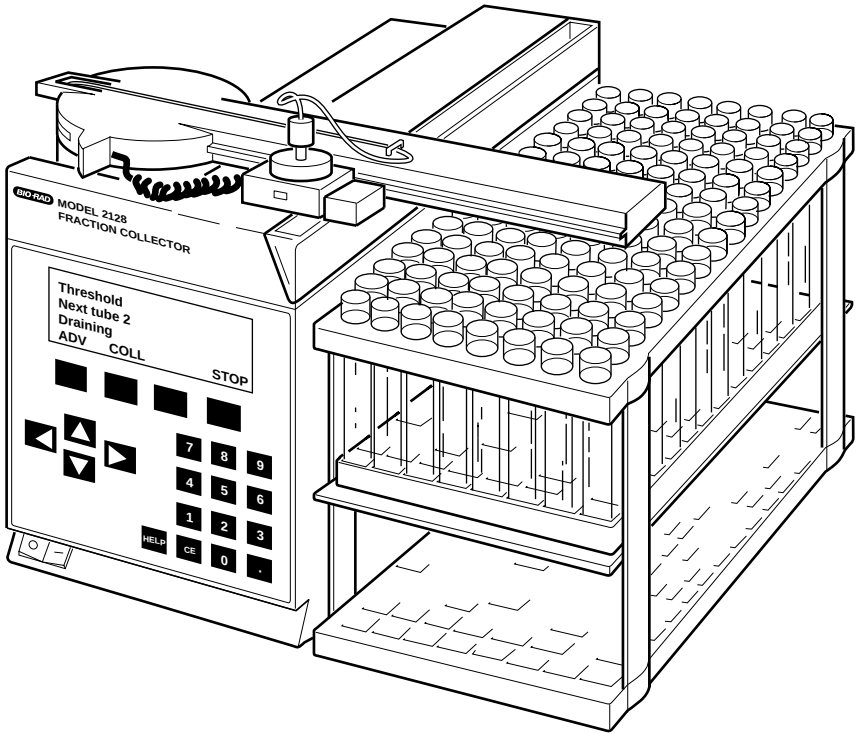


Figure 2-21. Model 2128 Fraction Collector

The following racks are available.

Table 2-13.
Model 2128 Racks Available

Rack Name (Software ID Number)	Tube Description	Capacity
Rack #1 (1)	12 and 13 mm diameter; 180 mm height (max.)	128 (16 X 8) tubes
Rack #2 (2)	16 to 18 mm diameter; 180 mm height (max.)	78 (13 X 6) tubes
Micro-Adapter (3)	Microplate (96 wells in 8 X 12 format)	3 plates (288 wells)
Micro-Adapter (4)	Microtubes (capless tubes required)	128 microtubes
Prep-Adapter (5)	Bottles of any size	10 bottles

The Model 2128 is controlled by the DuoFlow Controller via the Instrument Bus. See Chapter 3.8.3 for connection instructions.

The Model 2128 is plumbed to the DuoFlow system using 1/16” (1.6 mm) OD inlet tubing and either of the following fittings:

- To connect directly to the dispenser head, use 1/4-28 flat bottom fittings.
- To connect to the optional on-arm diverter valve, use the 10-32 nut and ferrule fittings supplied with the valve.

When the Model 2128 is configured in the Setup screen, collection schemes such as Collect All, Threshold, Collection Windows, and Threshold + Collection Windows (all with Delay volume, if required) automatically become available in the Protocol screen. The starting and ending tube numbers also may be specified. The Model 2128’s optional diverter valve mounts on the fraction collector’s arm and minimizes liquid spills during fraction advances. If this valve is used, it is automatically sensed by the system and hence is **not** configured by the user in the Setup screen.

This instrument is described in detail in its separate documentation.

2.7.4 Generic Fraction Collectors

A non-Bio-Rad collector may be used as an integral part of the DuoFlow system providing its tube advance function can be initiated by an active low TTL signal (>100 ms). Electrical connection from the collector is to pin 5 (Frac Advance) and pin 9 (ground) of the Workstation’s AUX connector. See the pin-out information in your collector manual.

With an optional SVT3-2 diverter valve configured as a *fraction collector diverter valve* in the Setup screen, the DuoFlow Controller will control all collection parameters including advanced features such as collection by Threshold and/or Collection Windows. If the optional valve is not used, then only Collect All is available.

Fraction advance marks to a Bio-Rad chart recorder are embedded on the UV signal from the Workstation.

2.8 SAMPLE LOADING OPTIONS

The BioLogic DuoFlow system supports several methods for sample loading. Typically a sample is loaded through a static fixed volume loop on the AVR7-3 inject valve. When large volume sample injections are required, the following options are available:

- Sample loading through the Workstation pumps: The SV5-4, SV3-2 or AVR9-8 valve may be used to load large sample volumes through the Workstation pumps. Refer to Sections 2.62 through 2.64 for the discussion of these valves.
- Sample loading through a DynaLoop sliding-piston loop: When used with an AVR7-3 inject valve, the DynaLoop may be used for large volume sample loading or repetitive injections of smaller volumes of sample. The DynaLoop comes in two sizes: 25 and 90 ml volume.
- Sample loading through an auxiliary pump, such as the EP-1 Econo pump (EP-1) or the Econo Gradient Pump (EGP). When used with an AVR7-3 inject valve, an EP-1 or EGP pump may be used for large volume sample injections. Placing an SV5-4 or AVR9-8 valve before the pump inlet allows loading of multiple samples.

2.8.1 DynaLoop Large Volume Sample Injection Loop

The DynaLoop, available in 25 ml and 90 ml sizes, is a sliding-piston dynamic loop for injection of a large sample volume or repetitive injections of smaller samples. The DynaLoop has a sliding piston that functions like a syringe. The loop is installed on the AVR7-3 in place of the static loop (see Figure 2-23). Sample is loaded into the DynaLoop's sample end connector through port #2 on the AVR7-3 valve and may be loaded with a syringe or auxiliary pumps (such as the Bio-Rad Econo Gradient Pump or EP-1 pump). The loading of the sample pushes the DynaLoop's sliding seal towards the buffer end connector. As solution flows into the buffer end of the DynaLoop it pushes the sample into the system.

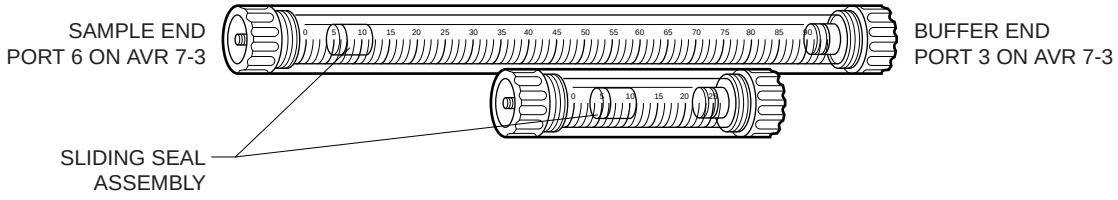


Figure 2-22. DynaLoop

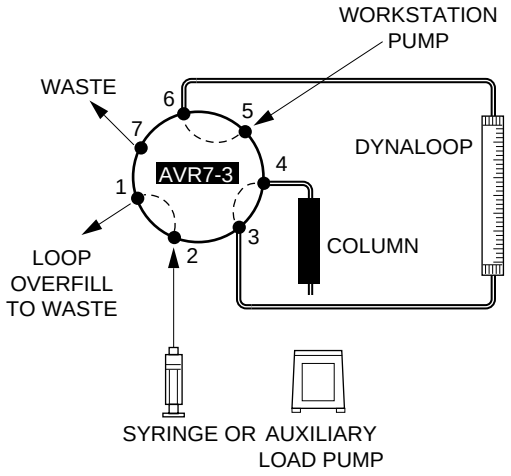


Figure 2-24. Plumbing the DynaLoop for use with an Inject Valve

When plumbed directly to the AVR7-3 inject valve, the DynaLoop functions just like a static sample loop. The sample end of the DynaLoop should be plumbed to port 3 and the buffer end to port 6 of the inject valve. The valve's operation and sample loading are controlled automatically, simplifying the sample injection process and insuring precise sample loading and gradient formation. The DynaLoop can be filled using a syringe or auxiliary pump.

Use a 1/4x28 to female Luer adapter between the sample load syringe and the AVR7-3 valve port 2 makes filling the loop much easier.

Consult the DynaLoop user's manual for additional information.

2.8.2 Model EP-1 Econo Pump

The Model EP-1 Econo pump is a two-channel, bi-directional, variable speed peristaltic pump for low-pressure chromatography. The EP-1 offers a full range of features to facilitate ease of use as a stand-alone pump or as an accessory to the DuoFlow System to load large volumes onto low pressure columns. This instrument is described in detail in its separate documentation. Features of the EP-1 include:

- The EP-1 has preset calibration for common tubing sizes and manual calibration for nonstandard size tubing.
- It controls various fraction collectors, including the BioFrac, Model 2110, and Model 2128 fraction collectors.
- It can control the run length with a programmable total run volume.
- It is coldroom compatible.

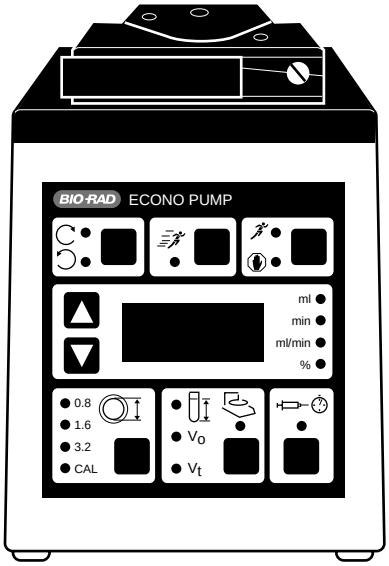


Figure 2-24. Model EP-1 Econo Pump

The EP-1 Econo pump is ideal for loading samples onto a low-pressure column. When using a low-pressure column, such as an Econo-Pac cartridge, remove the 40 psi backpressure regulator from the post-column position. Place it at the outlet of the Workstation pump. This will allow the check valves to properly seat and ensure pump flow performance.

The EP-1 Econo pump must contain firmware version 2.12 or higher to function with the BioLogic DuoFlow system. To confirm the firmware version, simultaneously press and hold down the Direction key and the down arrow key on the front panel of the EP-1 Econo pump. If the Econo pump does not have the correct firmware version, contact BioRad for information on how to upgrade the pump.

The EP-1 Econo pump receives start/stop signals only. The flow rate of the EP-1 Econo pump is set from the pump, and the flow rate is recorded in the yellow data entry boxes of the Load/Inject Sample dialog box.

The DuoFlow system starts/stops the Aux pump and cannot control the flow of the EP-1 Econo pump. The correct flow rate must be set at the EP-1 Econo pump.

Consult the EP-1 Econo pump user's manual for additional information.

2.8.3 EGP Econo Gradient Pump

The Econo Gradient Pump (EGP) is a two-channel, bi-directional, variable speed peristaltic pump for low-pressure chromatography and general laboratory use. The EGP has the following features:

- The EGP can be programmed to run a gradient.
- The EGP controls a splitter valve for stream splitting.
- The EGP may be used with most flexible tubing having an inner diameter less than or equal to 3.2 mm (1/8”), and a wall thickness of 1.0 mm or less, including Silicone, Tygon, and PharMed.
- Flow rates are 0.01-20 ml/min per channel, depending upon tubing ID.
- It has start/stop control of fraction collectors and chart recorders.
- It is coldroom compatible.

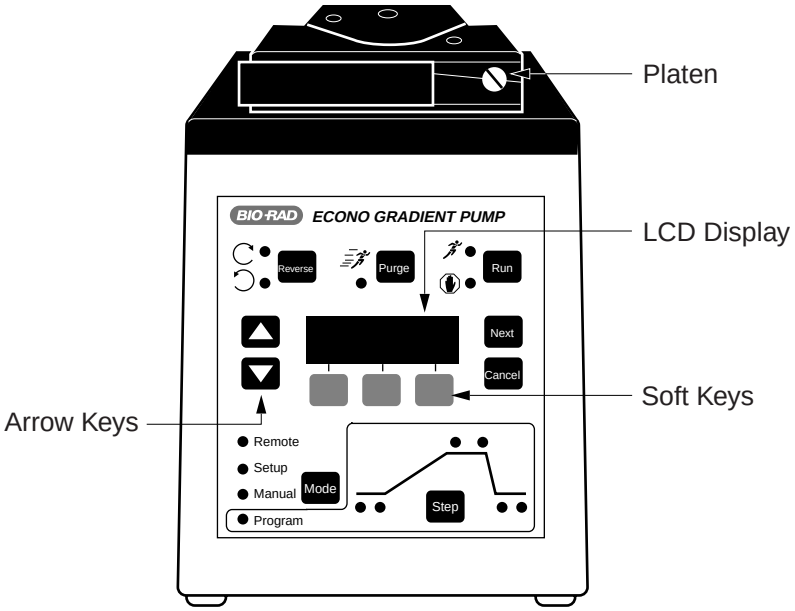


Figure 2-25. EGP Econo Gradient Pump

Consult the Econo gradient pump user manual for additional information.

2.8.4 Other Gradient Pumps

The BioLogic DuoFlow system sends a TTL signal to control an external pump. This signal is normally TTL high (5 volts). The DuoFlow system commands the external pump to run by holding Pin 4 "low". Any pump accepting this signal may be used with the DuoFlow system as a sample loading pump. Refer to your pump's separate documentation when connecting the pump to the DuoFlow system.

2.9 SYSTEM PERIPHERALS

A number of system peripherals are available for use with DuoFlow systems. The following system peripherals are discussed in this section:

- System rack
- Starter kit
- Fittings kit
- Fittings tightener
- Backpressure regulator
- SIM
- F10 and F40 Pump kits
- Chart recorders, including the Model 1327 chart recorder
- Uninterruptable power supply (UPS)
- Printers

2.9.1 System Rack

The BioLogic rack is an adaptable and durable racking system made of solvent-resistant polypropylene, stainless steel, and glass-filled nylon. The rack can stand alone or be placed directly on the Workstation and supports various columns and cartridges, valves, detection modules, buffer bottles, and peripheral equipment.

The rack should be assembled before placing it on the Workstation as described below (see Figure 2-26). Keep in mind that the illustration is only an example. Alternative rack arrangements include mounting the column clamps on the side of the rack to hold tall columns or setting up the rack to use only one or two trays.

1. Fit the tapered collars into the ringed grooves on the rods. The collars are tapered so that when they are attached to the rods and the rods are fitted into the holes at each end of the tray, the rods and collars serve as the legs of the tray. With this in mind, note the following guidelines:
   - For a 2 or 3-tray rack, you will need four long rods; for a 1-tray rack you will need just two long rods. The long rods have three grooves, one at each end and one in the middle. Attach the collars to one of the end grooves. It doesn't matter which of the end grooves you use. Attach the collars so that they flare out toward the end of the rod that will be placed at the bottom of the rack.
   - For a one tray rack, use the two short rods which have only one groove. Fit the collars onto the grooves so that the collars flare out toward the end of the rod that will be placed at the bottom of the rack.

2. Insert the rods into the holes at each corner of a tray. Insert the rods from *underneath* the tray such that they produce a firm fit in the holes.
3. For a 2 or 3-tray rack, attach sleeves to the middle of all four long vertical bars. Remember that the sleeves should be oriented so that the wide part of the taper is nearest the rod's bottom end.

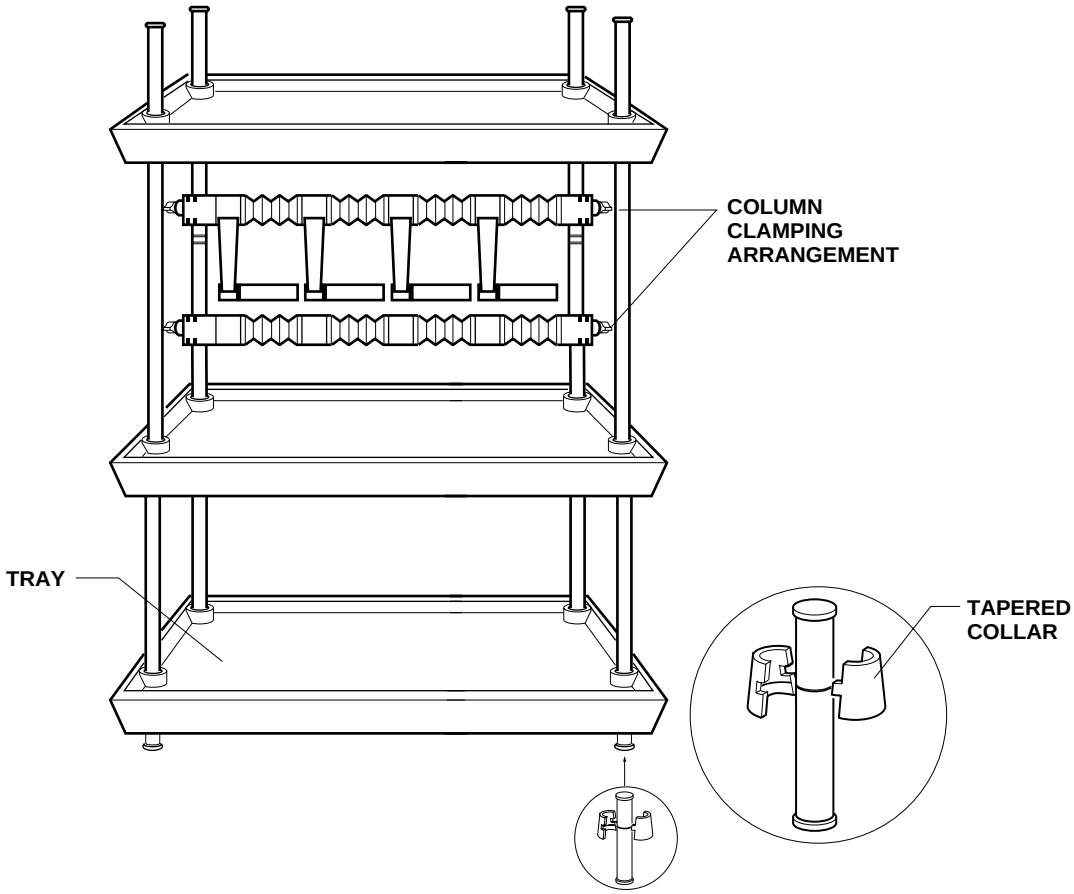


Figure 2-26. Rack Assembly

4. For a 2 or 3-tray rack, place the second tray on top of the four sleeves and press firmly to seat the tray. Repeat these instructions to add the upper tray.
5. Mount the 2-piece column clamping arrangement across the two long rods using the attached thumbscrews.
6. Two horizontal bars with rod clamps and an Allen wrench are provided with the system. These are used to hold devices, such as valves. Position them between any of the upright bars.
7. Remove the four green caps covering the holes at the four corners on top of the Workstation. Place the rack into the four corner holes.

2.9.2 Starter Kit

The Starter kit is included with each DuoFlow system. The kit includes step-by-step instructions for programming and running a separation of a premixed anion exchange standard containing equine myoglobin, conalbumin, chicken ovalbumin, and soybean trypsin inhibitor, using a 1.3 ml UNO Q1 Column.

The Starter kit includes the following items for running a separation:

- 50 ml of Buffer A, 250 mM Tris buffer pH 8.1 (10 X concentrate)
- 50 ml of Buffer B, 250 mM Tris buffer pH 8.1 plus 5.0 M NaCl (10 X concentrate)
- One vial of Anion Exchange Protein Standards (catalog number 125-0561)
- One 1-ml disposable sample injection syringe
- One 50 µl sample loop
- 50 ml of Maximizer solution A1 (10x concentrate)
- 50 ml of Maximizer solution A2 (10x concentrate)
- 50 ml of Maximizer solution B2 (2.5x concentrate)

The UNO Q1 Column (catalog number 720-0001) is not included with the Starter kit but is included with each DuoFlow system.

For a complete discussion of the Starter kit, refer to its separate documentation.

2.9.3 Fittings kit, including Tubing kit

The DuoFlow Fittings kit is included with all DuoFlow system. It contains the following components:

- PEEK and PTFE Tubing
- Tefzel Plugs, Adaptors, Unions, and Caps
- Tubing Cutter
- 3 ml Syringe and 10 ml Luer Slip Syringe
- Molded Bottle Caps
- BioLogic Fittings Tool
- Screwdriver
- PEEK F to M and F to F Luers
- Delrin Nuts
- Super Flangeless Ferrules
- F10 Tubing Kit

Optional tubing kits include the following:

- F10 Tubing Kit

  The F10 Tubing Kit includes precut 1/4-28 fitted PTFE, Tefzel, and 0.02” ID PEEK tubing for easy installation of a DuoFlow basic systems. An installation chart indicates suggested placement of each piece of labeled tubing (see Section 4.0).

  The kit includes: a length of PTFE tubing, 1/8” OD, for connecting solution bottles to the Workstation pumps A and B; a length of Tefzel tubing, 1/16” OD, for connecting AVR7-3 waste lines; and 8 additional pieces of PEEK, orange, 1/16” OD x 0.02” ID tubing, to connect the pump, mixer, column, detector, and other components.

- F40 Tubing kit

  The F40 Tubing kit is identical to the F10 kit (described previously), however, all orange PEEK tubing is replaced with PEEK, green, 1/16” OD x 0.03” ID tubing. The large bore tubing is designed for use with the higher flow rates when using the Maximizer or F40 Workstation pumps.

- Maximizer Tubing kit

  The tubing kit that accompanies the Maximizer for connecting the reagent bottles to the valves is composed of FEP PTFE, 1/8” OD x 0.062” ID. Each length of tubing is color coded to identify its connection site and solution.

  Inlet A1 uses red tubing and is used for acid solutions. Inlet A2 uses blue tubing and is used for base solutions. Inlet B1 uses yellow tubing and is used for water solutions. Inlet B2 uses green tubing and is used for salt solutions.

  The kit includes an installation chart.

- Maximizer Interconnect Tubing kit

  This kit contains cut and fitted tubing to connect the Workstation pumps A and B inlets to the Maximizer valves A and B outlets. There are two preformed PEEK 1/8” OD sets of tubing in this kit.

- pH Montior Tubing kit

  The pH monitor includes cut and fitted tubing to connect the pH flow cell to the Conductivity flow cell. There is a length of PEEK, orange, 1/16” OD x 0.02” ID tubing rated to 5000 psi and a length of PEEK, green, 1/16” OD x 0.03” ID tubing rated to 3000 psi for higher flow rates.

2.9.4 Fittings Tightener

The fittings tightener is designed to apply appropriate tightness to the nut, stainless steel lock ring, and the ferrule, when installing 1/4-28 fittings on the end of tubing. The flattened end of the lock ring should face towards the nut with the tapered end facing the tapered end of the brown ferrule. Place the tubing and fittings into the green fittings tightener. Do not allow the tubing to slip out of the ferrule. Tighten the fitting to seat the ferrule onto the tubing, but do not over-tighten. Once the fitting is made, it can be inserted into the port. Refer to instructions on next page.

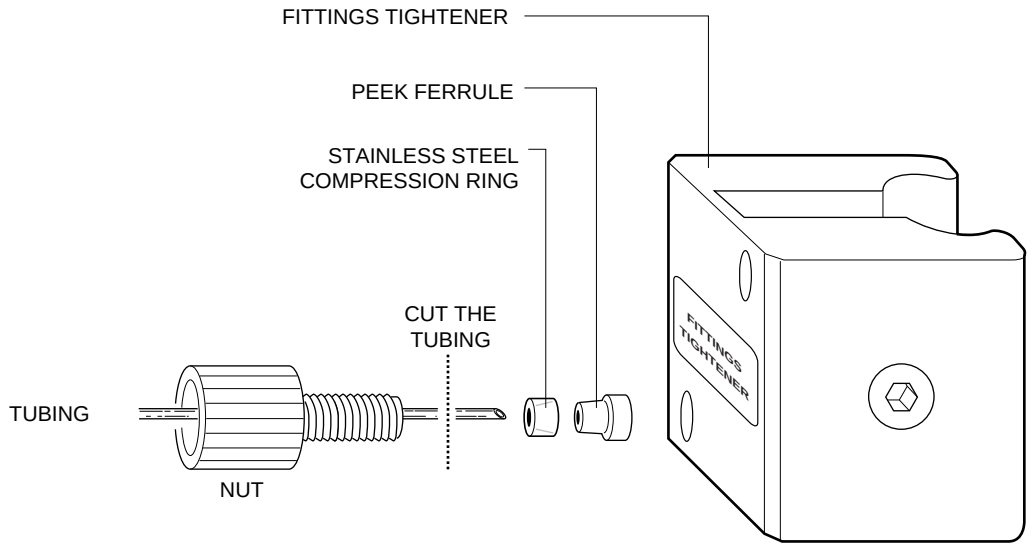


Figure 2-27. Making 1/4-28 Flat Bottom Fittings

The following procedure describes ferrule installation.

1. Slide the nut, stainless steel compression ring and the ferrule, in that order, onto the tubing as shown at left. The flattened end of the ring should face the nut. The tapered end of the ferrule should face the ring.
2. Allow tubing to extend slightly beyond the end of the ferrule.
3. Place the fitting into the green fittings tightener. Do not allow the tubing to slip out of the ferrule.
4. Insert the tubing and fitting into the fittings tool to tighten to seat the ferrule onto the tubing, do not overtighten.

2.9.5 Backpressure Regulator

The 40 psi backpressure regulator is used with flow rates below 10 ml/min. Plumb the backpressure regulator following the direction of the arrow.

The Backpressure Regulator helps eliminate bubble formation within the detector. As a solution is pumped through a column, the column exerts a backpressure that serves to keep any air bubbles in solution. Solution exiting the column returns to atmospheric pressure and air bubbles may form. As the bubbles pass through, or lodge in the detector flow cell they may cause artifacts on the baseline chromatogram that appear as spikes. This “outgassing” may be minimized by thoroughly degassing buffers and by placing a backpressure regulator after the Conductivity monitor. The backpressure from the regulator helps to keep the bubbles in solution.

When using low pressure columns such as an Econo-Pac® cartridge, plumb the 40 psi backpressure regulator between the Workstation pump outlet and the mixer. This aids in seating the check valves, preventing permanent damage to the cartridge or column.

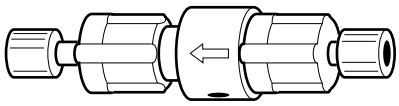


Figure 2-28. Backpressure Regulator

2.9.6 Signal Import Module (SIM)

The Signal Import Module digitizes an analog signal and transmits it to the DuoFlow Controller. It is used to import signals from non Bio-Rad detectors into the DuoFlow software.

- The SIM allows for connection of any pH probe and detector that outputs an analog signal between -2.5 Volts to +2.5 Volts. Instruments that may be connected in this way could include a variable wavelength UV detector, a refractive index detector, or a fluorescence detector.
- Two SIMs may be connected to a DuoFlow system.

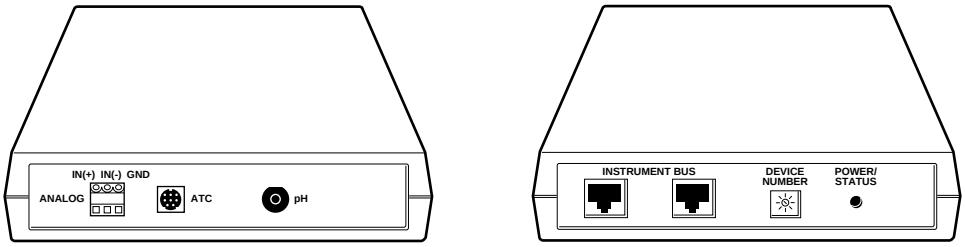


Figure 2-29. Signal Import Module (front and rear views)

The output from a pH electrode is connected to the pH connector (a BNC connector) on the SIM; the output from any other detector is connected at the 3-pin connector (+, -, gnd) of the SIM. The Automatic Temperature Compensation (ATC) connector is reserved for future upgrades and enhancements.

The instrument bus cable connects the SIM to the USB Bitbus Communicator. (Refer to Figures 2-29 and 2-30.) If a second SIM is to be used, one of the instrument bus cables is used to connect the two SIMs. The SIM is described in detail in its separate documentation. When a SIM is connected to the instrument bus, the USB Bitbus device must be connected to an external powersource (see Section 2.1.2).

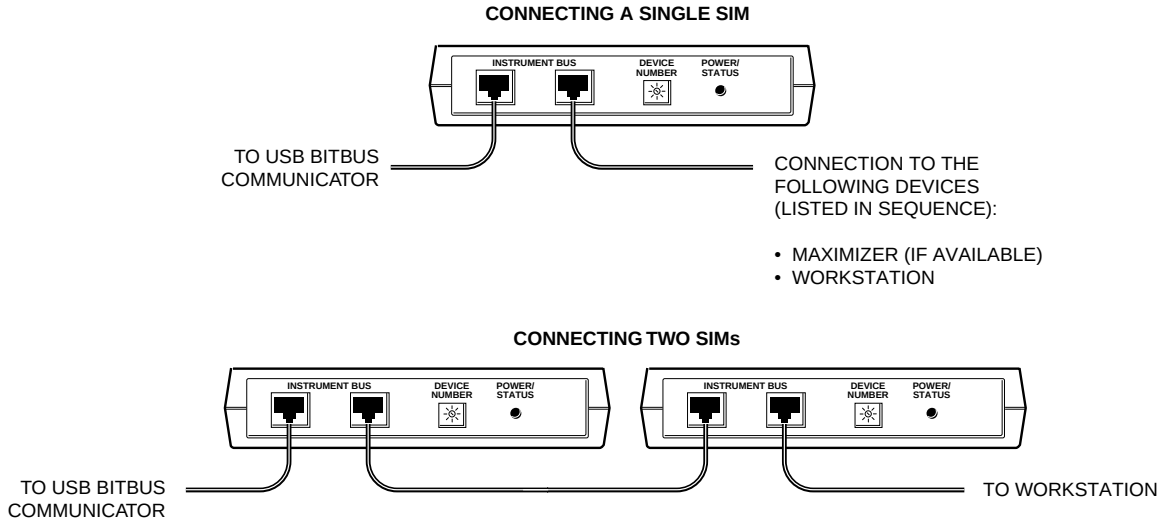


Figure 2-30. Cable Connections to the Signal Import Module

2.9.7 Pump Kits

The Workstation pumps are easily converted to either a F10 or F40 pumphead to expand functional pump flow rates as indicated in the table below.

Table 2-14.
Workstation Pump Configuaration Flow Rates

Workstation Pump	Flow Rate	Flow Rate with Maximizer
F10	0.01-10 ml/min	0.5-20 ml/min
F40	0.5-40 ml/min	1.0-80 ml/min

There are two kits, the F10 Pump kit and the F40 Pump kit. Each kit contains fully assembled pumpheads with seals and check valves installed.

The F10 Pump kit contains the following items:

- Two F10 pumpheads assembled with check valves, seals, and O-rings
- Four F10 piston assemblies
- Installation instructions

PEEK tubing, orange, 1/6” OD x 0.02 ID is supplied in the Fittings kit of the DuoFlow system

The F40 Pump kit contains the following items:

- Two F40 pumpheads assembled with check valves, seals, and O-rings
- Four F40 piston assemblies
- One 2 ml mixer barrel extender
- Two mixer O-rings
- One 2mm UV flow cell
- PEEK tubing, green, 1/16” OD x 0.03” ID (rated to 3000 psi)
- Installation instructions

For a complete discussion of the Pump kits, refer to the separate documentation supplied with the kits.

2.9.8 Model 1327 Chart Recorder

The Model 1327 chart recorder is a dual-pen chart recorder that is compatible with many detection devices. The chart recorder includes the following features:

- Two-channel, dual-pen capability.
- Compact in size (31 x 23 x 7.6 cm).
- Voltage ranges from 1 mV to 20V.
- Twelve chart speeds for recording methods.

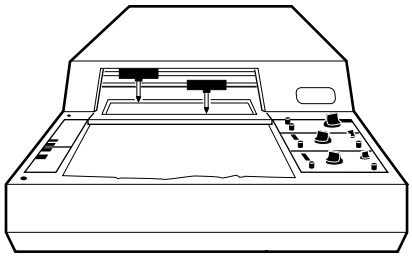


Figure 2-31. Model 1327 Chart Recorder

The DuoFlow Controller outputs the UV analog data signal, pen up/down, and Stop/Start commands to the Model 1327 chart recorder via the Workstation's UV Chart connector (cabled to the chart recorder using **System Cable 2** mini DIN to standard DIN). The Conductivity analog data signal is sent from the Workstation's Conductivity Chart (**Cond Chart**) connector to channel 2 of the chart recorder using **System Cable 4** (mini-DIN to banana plugs). The chart recorder should be set to all green settings (1V). The chart recorder is described in detail in its separate documentation.

Note: The chart speed is set at the recorder. The BioLogic DuoFlow does not control this function.

Event marks are recorded on the chart recorder for the following:

- Fraction Collector Advance: When the fraction collector advances to the next tube.
- A manual event mark from the Run screen. Pressing the **Event Mark** button on the Run screen allows you to "mark" events as they happen.

Non-Bio-Rad UV detectors: If the Model 1327 chart recorder is used in conjunction with a SIM and a third-party detector to replace the DuoFlow UV Detector, **System Cable 20** should be used to control the recorder. Channel 1 signals should be sent directly from the third-party detector using a bare wires-to-banana plug cable. In this case, the appropriate input voltage range must be selected on the recorder. Refer to the documentation for your non-Bio-Rad UV detector.

2.9.9 Generic Chart Recorders

A non-Bio-Rad chart recorder may be used as an integral part of the DuoFlow system. If the DuoFlow system's UV detector is used, a mini-DIN to breakout cable (**System Cable 7**) must connect the Workstation's UV Chart connector to the chart recorder. Pen Up/Down and Start/Stop functions are available providing the control polarity of the generic recorder is compatible. See the pin-out information in your chart recorder manual.

Conductivity signals require a Bio-Rad mini-DIN to banana plug cable (**System Cable 4**).

Set the chart recorder's input signal voltage to 1 V for both BioLogic DuoFlow UV detector and Conductivity monitor signals. Chart speed is set at the recorder itself, it is not controlled by the DuoFlow system.

2.9.10 Uninterruptible Power Supply (UPS)

A UPS may be required in laboratory environments that experience power outages or where the quality of power varies. Bio-Rad can supply a UPS in both 110 V and 220 V configurations. For questions about this UPS, consult your local Bio-Rad representative.

2.9.11 Printers

Any Windows® 2000 compatible printer may be used with the DuoFlow systems. The printer must include a connection cable and a printer driver.

To install a printer driver, exit the DuoFlow software. If the printer driver disk is available, follow the instructions that are included with that disk. If the printer driver disk is not available, click on the Windows® **Start** button and from **Settings,** select **Control Panel**.

In the Control Panel, double-click on the **Printers** icon to open the Printers window. Then double-click on the **Add Printer** icon and follow the on-line instructions. If your printer does not appear in the list of printers available, contact the printer manufacturer for a recommendation on which driver to use.

2.10 COLUMNS AND COLUMN FITTINGS

Bio-Rad offers a variety of column chemistries and formats. The following pages provide a summary of the different column types and column fittings for use with BioLogic DuoFlow systems. Bio-Rad columns for the DuoFlow use the following matrices:

- UNO™ columns use the new Continuous Bed matrix which contains an advanced polymer matrix that is completely homogeneous. These columns are superior to beaded supports in resolution, binding capacity, speed, and value.
- Bio-Scale chemistries are based on 10 µm Macro-Prep® supports. They are offered in various sizes and allow for scale-up separation and purification. These medium-pressure columns are ideally suited for use on the DuoFlow system.
- The Econo-Pac cartridge chemistries are based on 50 µm Macro-Prep® supports and are ideal for first step purification.

2.10.1 Anion Exchange: Q Strong Anion Exchange

The Q strong anion exchanger chemistry is available in the following formats:

- **UNO Q Biochromatography Columns:** These columns are designed to handle separations at high flow rates with low back pressure. Instead of a traditional bed of packed beads or particles, each column contains an advanced polymer matrix, called the Continuous Bed matrix, which is nonporous and homogeneous. The matrix is designed to maximize resolution, binding capacity, and speed. Refer to bulletins 2116 and 1946.
- **Bio-Scale Q Prepacked Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® supports. Refer to bulletins 1880, 1946, and 2079.
- **Econo-Pac High Q Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletin 1946.
- **Macro-Prep® High Q Support:** This is a strong anion exchanger containing quaternary amine functional groups with a 50 µm particle size. It is ideal for rapid purification of acidic and neutral proteins and peptides. Refer to bulletins 1840 (A-100), 1917, and 1985.

2.10.2 Cation Exchange: S Strong Cation Exchange

The S strong cation exchanger chemistry is available in the following formats:

- **UNO S Biochromatography Columns:** These columns are designed to handle separations at high flow rates with low back pressure. Instead of a traditional bed of packed beads or particles, each column contains an advanced polymer matrix, called the Continuous Bed matrix, which is nonporous and homogeneous. The matrix is designed to maximize resolution, binding capacity, and speed. Refer to bulletins 2116 and 1946.
- **Bio-Scale S Pre-packed Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® 50 µm supports. Refer to bulletins 1881, 1946, and 2079.

- **Econo-Pac High S Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletin 1985.
- **Macro-Prep® High S Support:** This is a strong cation exchanger containing sulfonic acid functional groups with a 50 µm particle size. It is ideal for purification of basic and neutral proteins and peptides. Refer to bulletins 1840 (A-200), 1917, and 2079.

2.10.3 Anion Exchange: DEAE Weak Anion Exchange

The DEAE weak anion exchanger chemistry is available in the following formats:

- **Bio-Scale DEAE Prepacked Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® 10 µm supports. Refer to bulletins 1930, 1946, and 2079.
- **Econo-Pac DEAE Blue Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletin 1946.
- **Macro-Prep® DEAE Support:** This is a weak anion exchanger containing diethylaminoethyl functional groups with a 50 µm particle size. It is ideal for purification of acidic and neutral proteins and peptides. Refer to bulletin 1840 (A-400).

2.10.4 Cation Exchange: Carboxy Methyl (CM) Weak Cation Exchange

The CM weak cation exchanger chemistry is available in the following formats:

- **Econo-Pac CM Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports.

2.10.5 Ceramic Hydroxyapatite (CHT)

The CHT chemistry is available in the following formats:

- **Bio-Scale CHT Type I Prepacked Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® 10 µm supports. Refer to bulletins 1929, 1946, 2079, and 2156.
- **Econo-Pac CHT-II Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 20 µm Macro-Prep® supports. Refer to bulletin 1946.

- **Macro-Prep® CHT Support:** Macro-Prep® ceramic hydroxyapatite (CHT) overcomes the physical and chemical instability of crystalline hydroxyapatite and is available in two types. Type I has a high protein binding capacity and better binding capacity for acidic proteins. Type II is better suited for proteins that elute early and for nucleic acids. Refer to bulletins 1842 (C-100), 1927, 1971, and 2156.

2.10.6 Size Exclusion Chromatography (SEC)

The SEC supports are available in the following formats:

- **HPLC SEC Columns:** Bio-Sil and Bio-Select 5 µm silica-based columns separate compounds by the mechanism of size exclusion. The technique is based on diffusion in and around highly porous spherical silica beads. The columns are recommended for the separation of peptides, proteins, and nucleic acids, for desalting or buffer exchange, and for molecular weight or molecular constant determination. Columns are available in both stainless steel (Bio-Sil columns) and biocompatible PEEK plastic hardware (Bio-Select columns). Each column is shipped with a free vial of Bio-Rad's protein standards. Refer to bulletins 1737 and 1946.
- **Econo-Pac P6 Low Pressure Chromatography Cartridges:** This 5 ml cartridge is used for desalting and buffer exchange. Refer to bulletin 1946.

2.10.7 High Pressure Reversed Phase Columns

The following type of column is available:

- **Hi-Pore reversed phase columns:** These columns are commonly used for the purification and analysis of small proteins (<50 kd), peptides, oligonucleotides, and amino acids. Refer to bulletin 1946.

2.10.8 Hydrophobic Interaction Chromatography (HIC)

The HIC chemistry is available in the following formats:

- **Econo-Pac t-Butyl HIC Low Pressure Chromatography Cartridges:** These cartridges are available in a 5 ml format to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletins 1946 and 2079.
- **Econo-Pac HIC Low Pressure Chromatography Cartridges:** HIC is offered in both methyl and t-butyl chemistries. These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep supports. Refer to bulletin 1946.
- **Macro-Prep® HIC Support:** HIC is offered in both methyl and t-butyl chemistries. The Macro-Prep® methyl HIC support is ideal for purification of proteins with strongly hydrophobic regions. The Macro-Prep® t-butyl HIC support is ideal for purification of proteins with few or weakly hydrophobic regions. Refer to bulletin 1841.

2.10.9 Affinity Chromatography

Affinity chromatography is available in the following formats:

- **Econo-Pac Protein A Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for monoclonal antibody purification. Refer to bulletin 1946.
- **Econo-Pac Blue and DEAE Blue Low Pressure Chromatography Cartridges:** These chemistries are available in a 5 ml format and are recommended for albumin and protease removal. Refer to bulletin 1946.

2.10.10 Empty Columns

The following columns are available:

- **Bio-Scale MT empty columns:** These columns allow for easy packing of a chromatographic media, bed height adjustment, sample application, and equilibration. The availability of four column sizes (2, 5, 10, and 20 ml) allows easy scale-up of separation and purification protocols. Request bulletin 1970.
- **Glass Econo-Column columns**: Econo-Column chromatography columns are the standard for high quality, affordable low pressure chromatography columns. They accept both Econo-Column funnels and flow adapters.

2.10.11 Column Fittings

Table 2-15.
Columns and Column Fittings

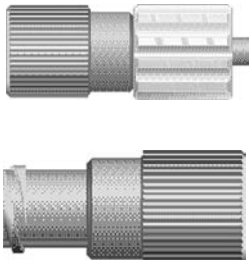
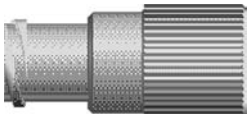
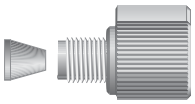
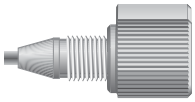
Column Type	Description	Fittings Required
Econo-Pac® Cartridge	The Econo-Pac cartridge has one male and one female luer-lock fitting. To connect it to the BioLogic system use 1/4-28 to male and 1/4-28 to female luer adapters, provided in the kit shown to the right. One set is included in the Fittings kit.	**Catalog # 732-0113** Econo-Pac® Cartridge to Fittings Kit 
Econo-Column®/ Low Pressure Columns and flow adapters	Econo-Columns have two male luer-lock fittings. To connect to the system, use female luer to 1/4-28 adapters. If a flow adapter with flexible tubing is to be used, attach the barbed-to-male luer fitting (included in this kit), then connect the female luer to 1/4-28 adapter. If the column adapter uses PTFE tubing, attach the small length of 1/16” (1.6 mm) ID Tygon® tubing and insert the barbed male luer fitting into open end. Five cm of Tygon tubing is included in this kit. Connect as above.	**Catalog # 750-0565** Econo-Column® to BioLogic System Fittings Kit  This kit includes all the parts needed to connect Econo-Columns and flow adapters to the BioLogic DuoFlow system.
FPLC® Column	Pharmacia’s FPLC columns use M-6 fittings. To connect to the system, use 1/4-28 to M6 adapter union. Two of these are supplied with the Fittings kit.	**Catalog # 750-0561** Union, 1/4-28 to M6 

Table 2-15. (continued)
Columns and Column Fittings

Column Type	Description	Fittings Required
HPLC Column, or **Pharmacia's RESOURCE™ Column**	These columns accept 10/32 nuts. To connect this type of column to the system, use two 1/4-28 to 10-32 adapters.	**Catalog # 750-0564** HPLC Column to BioLogic System Adapters 
UNO™ Column to FPLC system	This kit includes two nuts and four ferrules to connect an UNO column to an FPLC system.	**Catalog #750-0567** M6 Fittings Kit 
UNO™ Column to HPLC system	This kit includes two nuts and four ferrules to connect an UNO column to an HPLC system.	**Catalog #750-0568** 10-32 Fittings Kit 

3.0 SYSTEM SETUP

The modular design of BioLogic DuoFlow systems permits you to arrange the system to best meet application and space requirements. Each of the following subsections discuss how to set up the different components. Setup of each component is the same, regardless of the system you have.

The Workstation and the Maximizer have many rear panel connectors that are identical.The conductivity monitor must be connected to the Maximizer.

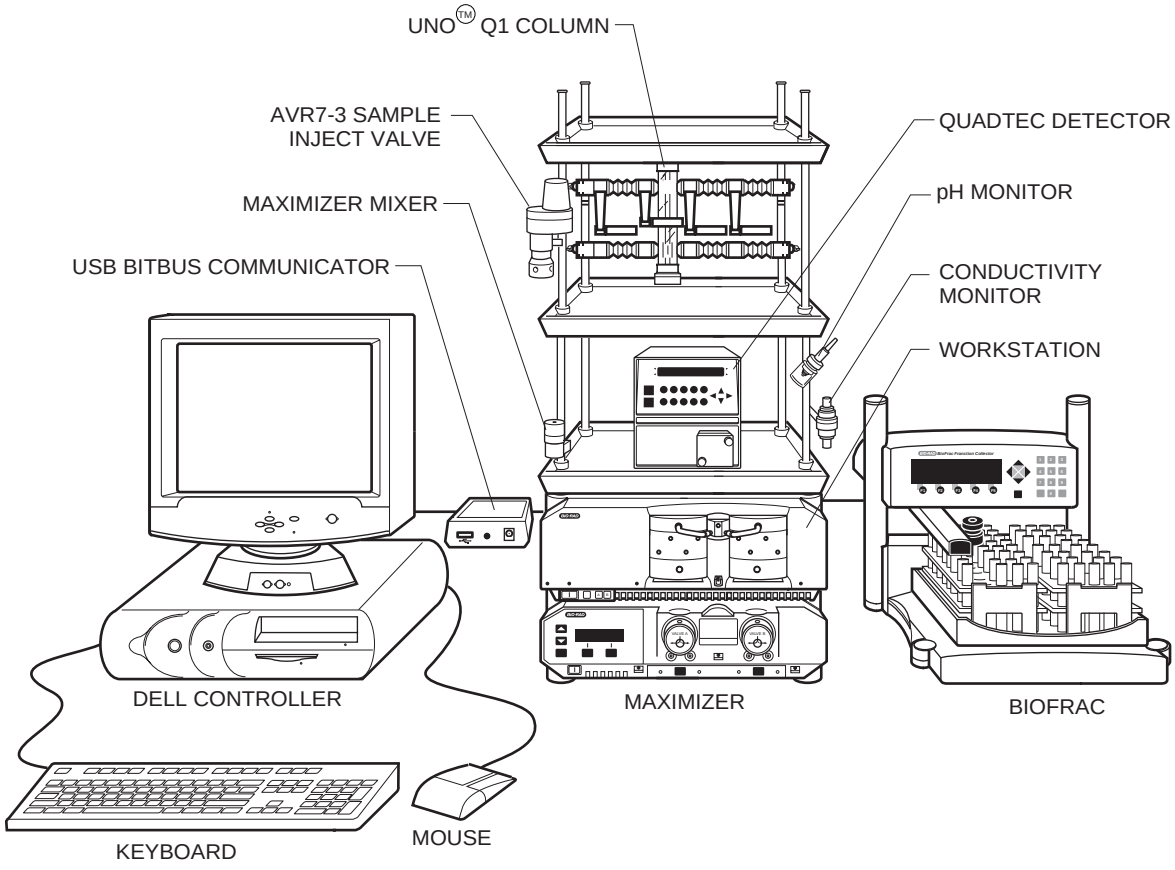


Figure 3-1. Example of a DuoFlow Pathfinder System Configuration

The compact design of the Workstation and rack are ideal for laboratories with limited space or for systems that must go into a cold box or cold room. For these instances the Controller can be placed away from the Workstation (see Figure 3-2 below). Bus communication cables of lengths up to 100 feet can be purchased from Bio-Rad, and USB cables can be purchased from any computer supply store.

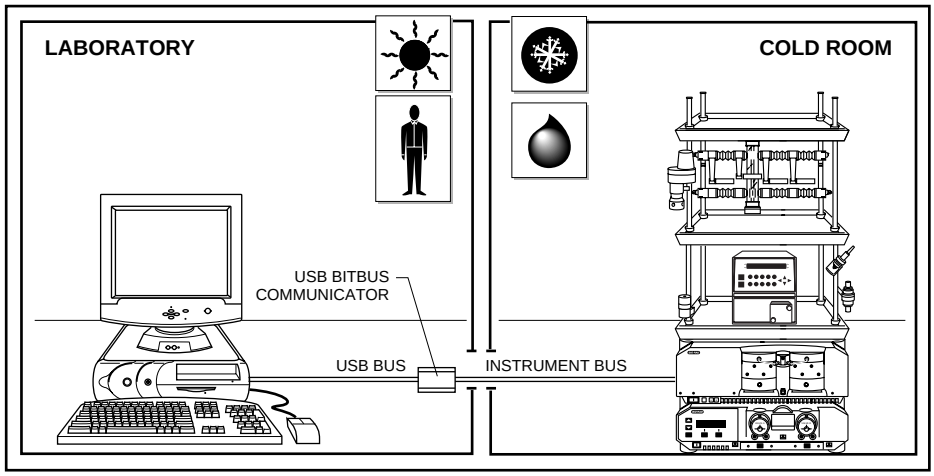


Figure 3-2. DuoFlow Pathfinder Setup in Non-Condensing Environment

3.1 CONTROLLER CABLE CONNECTIONS

The Controller consists of a monitor, computer, keyboard, and mouse. Bio-Rad provides a Dell computer for use as a Controller.

Detailed instructions for setting up your Controller are provided in the separate documentation provided with your Controller.

1. Place the Controller so that the back side is facing you. Connect the monitor, keyboard, and mouse to the computer.
2. Connect the power cables to the computer and monitor. If the computer has a second power outlet, use it to plug in the monitor power cable. **Do not turn on power to these devices yet.**

3.2 USB BITBUS COMMUNICATOR CABLE CONNECTIONS

The USB Bitbus Communicator allows communication between the Controller and the rest of the DuoFlow system. To connect the USB Bitbus Communicator:

1. Connect System Cable **31** from the USB Bitbus Communicator to the computer USB connector. Refer to the illustration below.
2. If a Signal Import Module (SIM) is to be used with the system (see lower half of Figure 3-3).
   a. Connect the USB Bitbus Communicator power adapter (catalog number 760-2034).
   b. Set the Power Select switch to External.

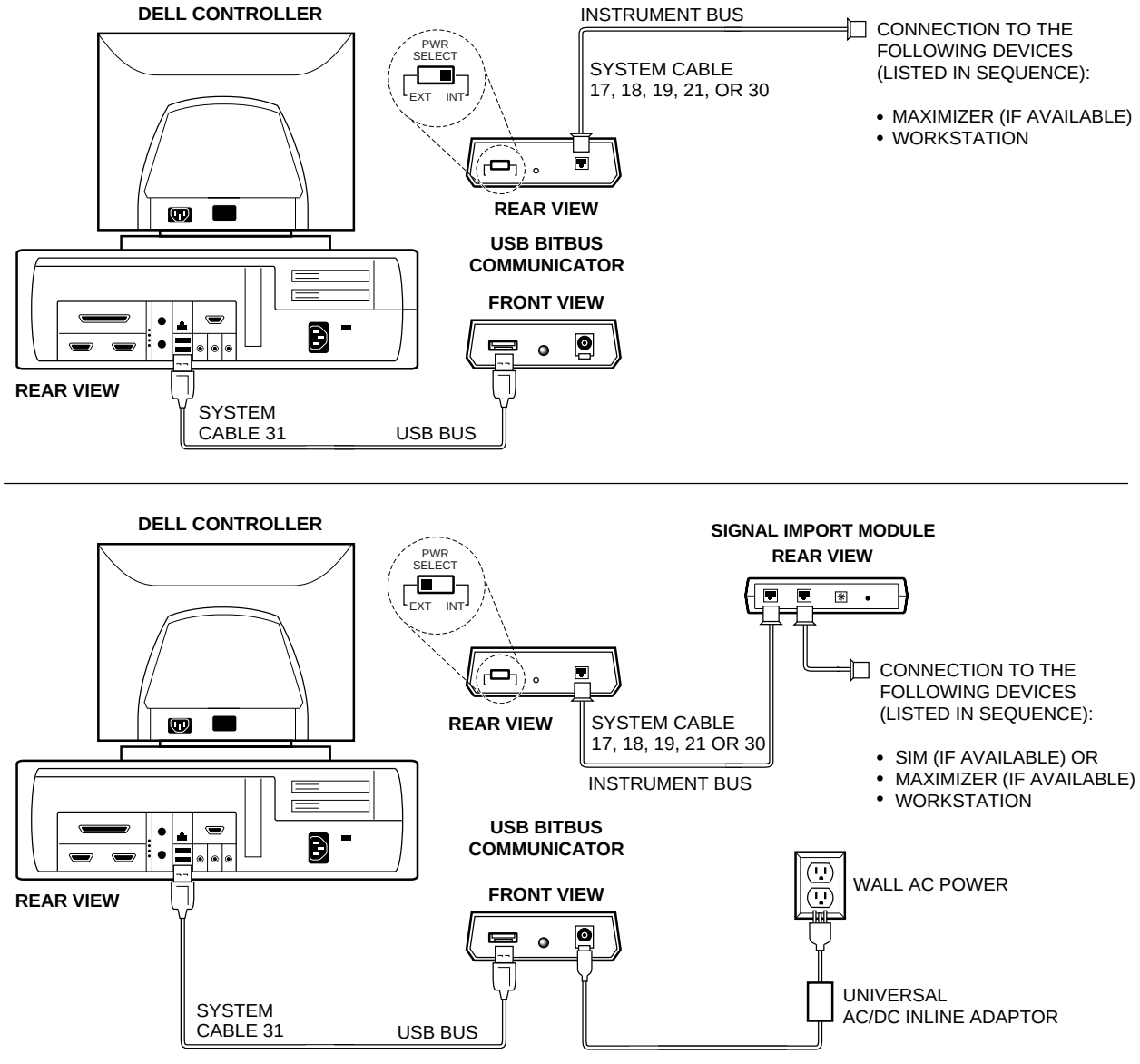


Figure 3-3. USB Bitbus Communicator Cabling

3.3 WORKSTATION CABLE CONNECTIONS

1. Confirm the voltage setting for the Workstation power supply. A red switch on the back of the Workstation allows you to switch between 110 VAC and 240 VAC.
2. Connect the power cable to the Workstation. **Do not turn on this device yet.**

3.3.1 Systems *without* a Maximizer

If the Maximizer is NOT to be part of the system, follow the procedure below to connect the Workstation to the USB Bitbus Communicator.

1. Note the two connectors marked “Instr Bus.” These connectors are identical: either may be used when connecting bus communication cables.
2. Select a System Cable of sufficient length to reach the USB Bitbus Communicator. System Cables 17, 18, 19, 21, and 30 differ only in their lengths.

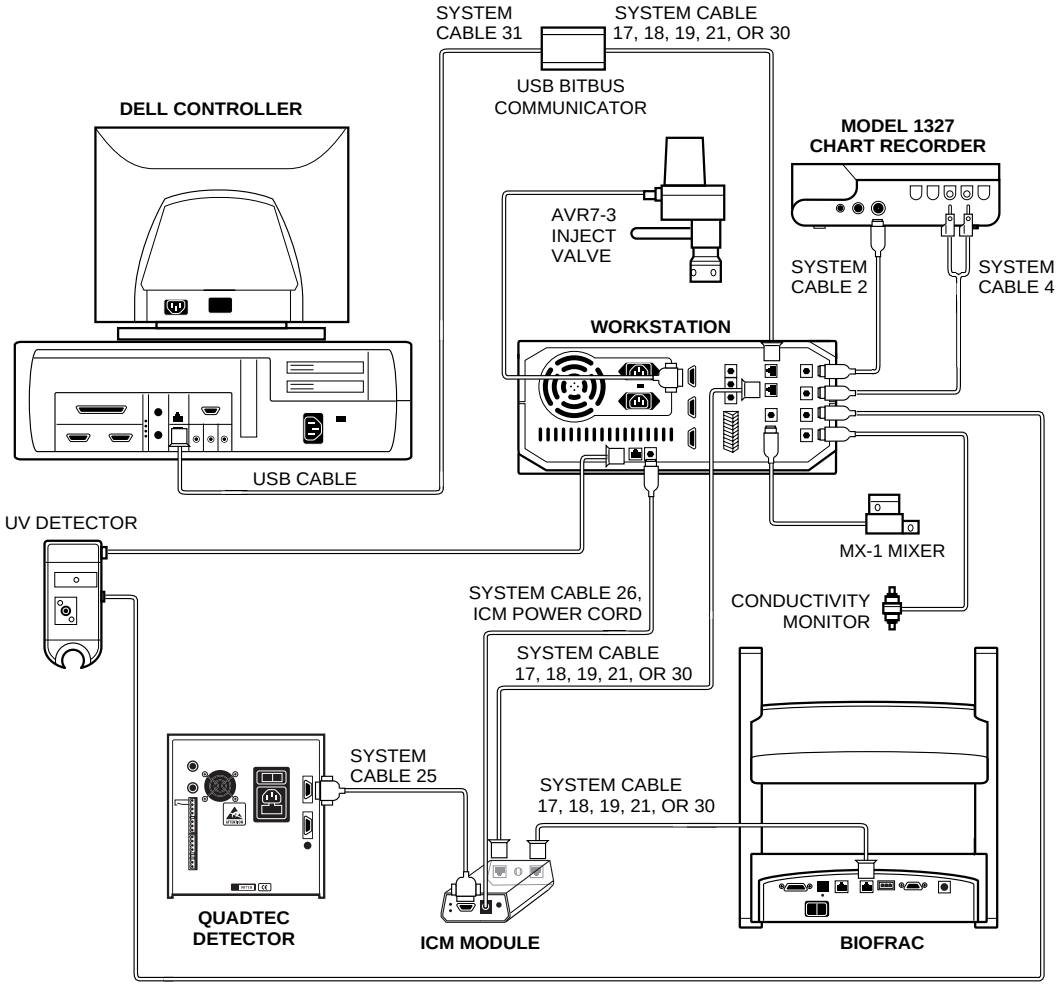


Figure 3-4. System Cable Connections (without Maximizer)

3.3.2 Systems *with* a Maximizer

If the Maximizer is to be part of the system, follow the procedure below to connect the Maximizer to the Workstation and the USB Bitbus Communicator.

1. Place the Workstation on top of the Maximizer.
2. Note the two connectors marked “Instr Bus.” These connectors are identical: either may be used when connecting instrument bus cables.
3. With the back side of the Workstation and Maximizer facing you, connect **System Cable 30** between the Workstation and the Maximizer.
4. Select a System Cable of sufficient length to reach the USB Bitbus Communicator. System Cables 17, 18, 19, 21, and 30 are different only in their lengths.
5. Connect the power cable to the Maximizer. **Do not turn on this device yet.**

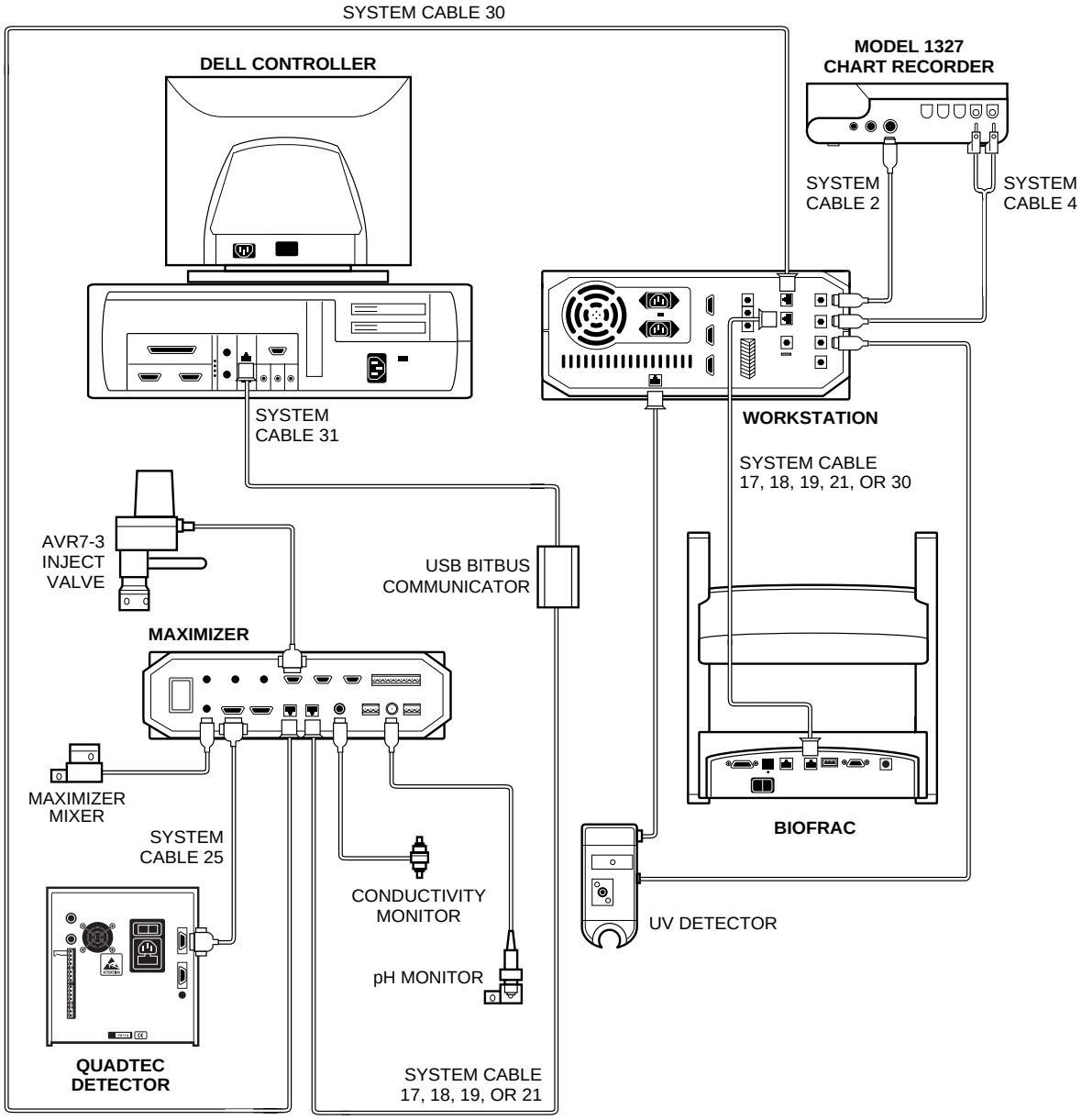


Figure 3-5. System Cable Connections (with Maximizer)

3.4 SYSTEM RACK SETUP

Assemble the system rack before placing it on the Workstation. Detailed discussion of system rack assembly is provided in Section 2.9.1. Keep in mind that the illustration is only an example. Alternative rack arrangements include setting up the rack to use only one or two trays.

To mount the system rack on the Workstation, remove the four green caps covering the holes at the four corners on top of the Workstation. Place the rack into the four corner holes.

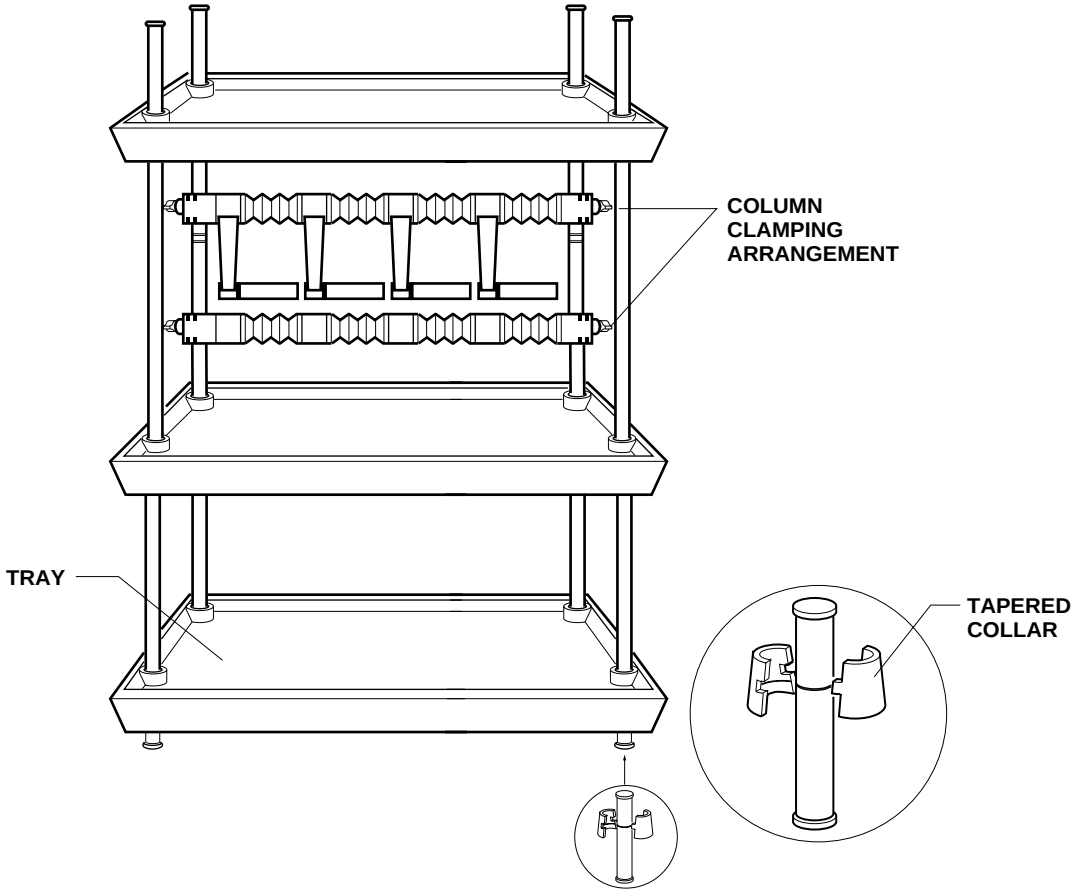


Figure 3-6. Rack Assembly

3.5 MIXERS

Two mixers have been designed for use with the DuoFlow system: the MX-1 mixer and the higher capacity Maximizer mixer. The mixers are shipped pre-assembled with their mixer barrel. The mixer barrel may be removed or replaced increasing or decreasing the internal volume to provide the mixer capacity appropriate to the flow rate. Refer to table 3.1 when selecting a mixer volume.

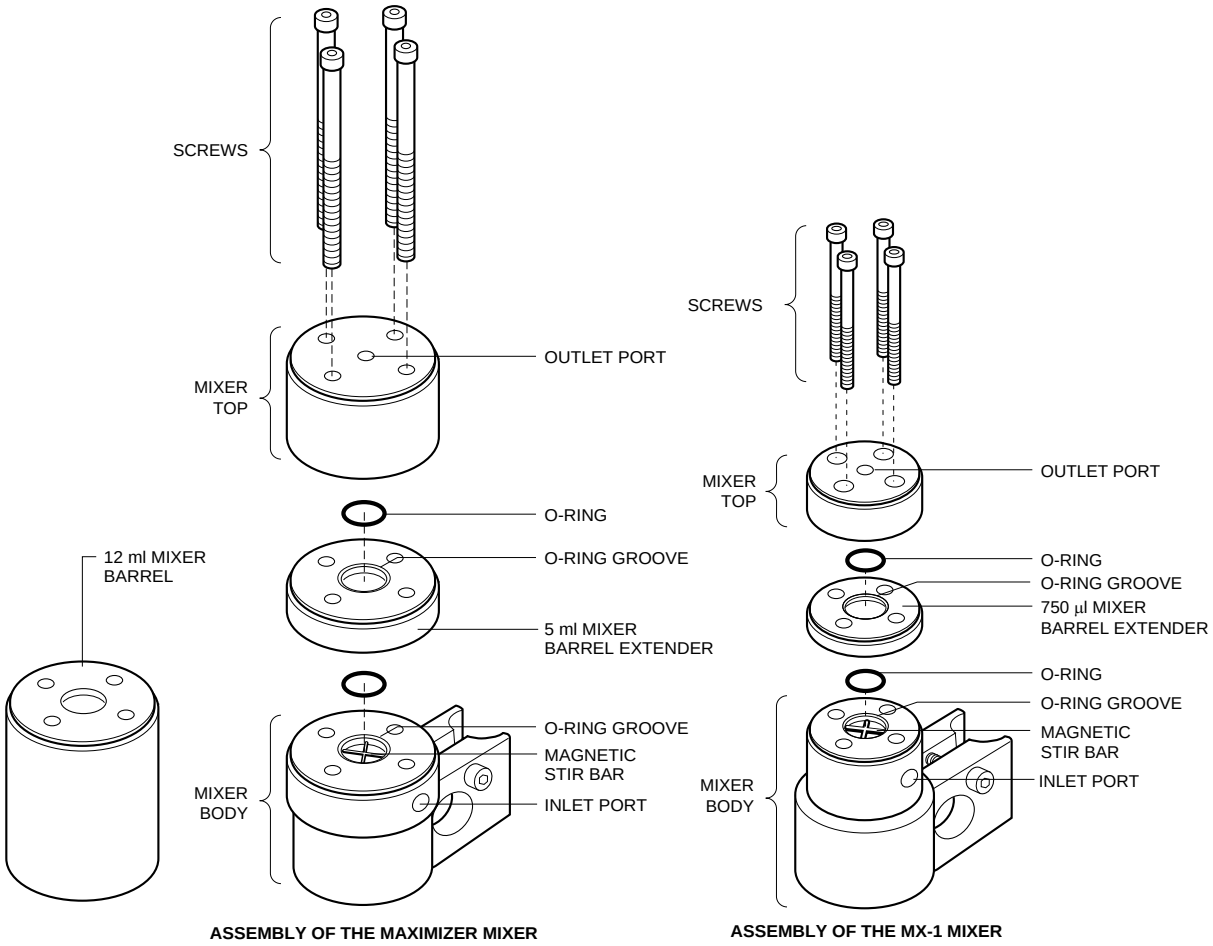


Figure 3-7. Mixers

Table 3-1.
Mixer Flow Rates

Flow Rate	Barrel Extension	Capacity	Assembly Screws
MX-1 Mixer (for use without Maximizer)			
less than 1ml/min	none	263 µl	10-32 x 5/8” (1.6 cm)
1 to 10 ml/min	750 µl mixer barrel extender	750 µl	10-32 x 7/8” (2.2 cm)
10 to 40 ml/min	2 ml mixer barrel extender	2 ml	10-32 x 1-1/2” (3.8 cm)
Maximizer Mixer (for use with Maximizer)			
0.5 to 10 ml/min	none	750 µl	1/4-20 x 1/2” (1.3 cm)
10 to 40 ml/min	5 ml mixer barrel extender	5 ml	1/4-20 x 1-1/2” (3.8 cm)
40 to 80 ml/min	12 ml mixer barrel extender	12 ml	1/4-20 x 3-1/4” (8.2 cm)

To attach the Mixer to the rack:

1. Confirm that the selected mixer and its capacity volume is appropriate for the flow rate. If it is not, refer to the previous chapter for the procedure for changing the mixer capacity.
2. Attach the mixer to a vertical bar using its rod clamp. The mixer should be positioned between the pump and the AVR7-3 inject valve. Attach the mixer to the rack so that the mixer outlet port faces upward.
3. Connect the mixer signal cable (mini-DIN connector) to the connector marked Mixer on the rear of the Maximizer, if available. Otherwise, connect to the Workstation. Refer to Figures 3-4 and 3-5.
4. Plug the unused inlet port using the plug provided.

3.6 DETECTION SYSTEM CONNECTIONS

This section discusses how to connect the UV detector, the Conductivity monitor, the QuadTec detector, and non-Bio-Rad UV detectors.

3.6.1 UV Detector and Conductivity Monitor

Two flow cells are available for use with the UV detector.

- Analytical 5 mm flow cell for high resolution protein chromatography applications and low flow rates. It has a path length of 5 mm for maximum sensitivity and a volume of only 16 µl. It can be used with flow rates from 0.1 to 10 ml/min.
- Preparative 2 mm flow cell for work not requiring high sensitivity, or when working with high protein concentrations, and for flow rates greater than 10 ml/min. It has a path length of 2 mm and a volume of 30 µl.

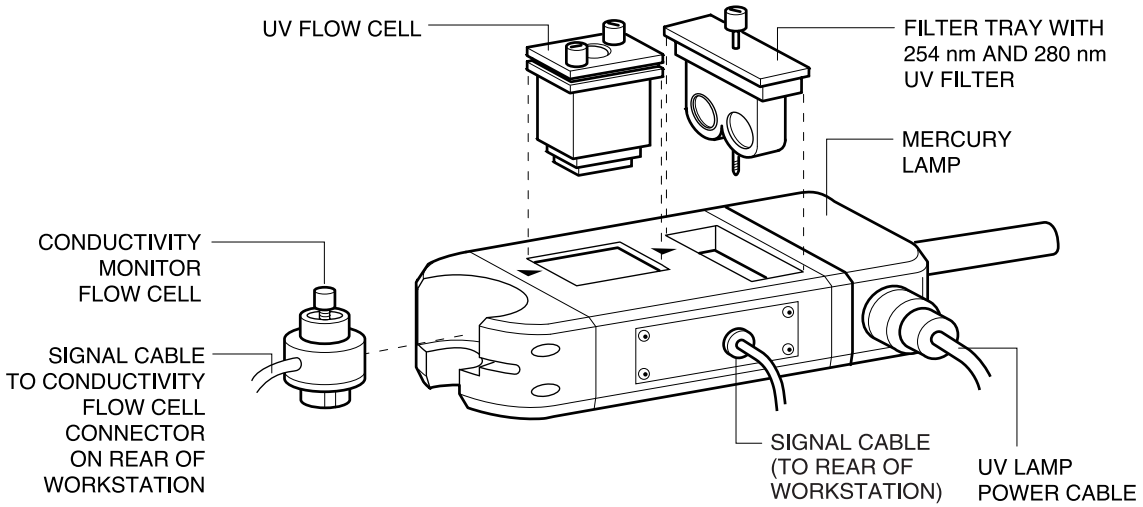


Figure 3-8. UV Detector and Conductivity Monitor

To attach the UV Detector and Conductivity Monitor to the rack:

1. Using the information provided above, confirm that the UV flow cell capacity volume is appropriate for the flow rate. Complete discussion of the UV detector and conductivity monitor is provided in Section 2.5, including the procedure for changing the UV flow cell.
2. Using the rod clamps, attach the UV detector to a vertical or horizontal bar close to the column outlet.
3. Connect the power cable (square connector) from the UV detector lamp into the connector marked **UV Lamp** on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
4. Connect the UV detector signal cable (mini-DIN connector) to the connector marked **UV Optics** on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
5. Connect the Conductivity monitor's combined power and signal cable (mini-DIN connector) to the connector marked **Cond. Flow cell** on the rear of the Maximizer, if available. Otherwise, connect it to the Workstation. Refer to Figures 3-4 and 3-5.
6. The Conductivity monitor is designed to be held in the circular notch of the optics bench, but may be placed anywhere in the fluid path.

3.6.2 QuadTec UV/Vis Detector

The QuadTec detector is shipped with a “dummy” flow cell installed. Before operating the detector, you need to install the biocompatible 3mm PEEK flow cell. For complete discussion of the QuadTec detector, including the procedure for installing the flow cell, refer to the QuadTec Instruction Manual.

1. Make sure the detector power is OFF.
2. Loosen the two knurled screws on the front of the flow cell and gently pull them out. This allows the flow cell housing to slide out.
3. Remove the dummy cell by gently pulling it upward. Save the dummy cell in a secure place. It is required for measuring signal and reference output values for the lamps. See Section 5.1, Checking the Status of D2 Lamp, in the QuadTec instruction manual for instruction on recording signal and reference values.
4. Insert the new flow cell and make sure that the engraved specifications point towards the user and that the fixing hole on the back side of the cell meets the corresponding metal pin of the detector's housing.
5. Slide the complete system towards the detector, insert the two knurled screws, and tighten them by hand.
6. Attach the 10-32 Fingertight fittings and tubing to the inlet and outlet ports and use a syringe to rinse the flow cell with 10-20 ml of 100% analytical grade methanol, followed by 5 ml distilled deionizied (DDI) water.
7. Connect the QuadTec to the DuoFlow system. If the Maximizer is to be used, the QuadTec should be connected to the Maximizer rear panel Com 1 connector using System Cable 25. If the Maximizer is not in use, connect the QuadTec via the Instrument Control Module (ICM), as discussed on page 3-11.

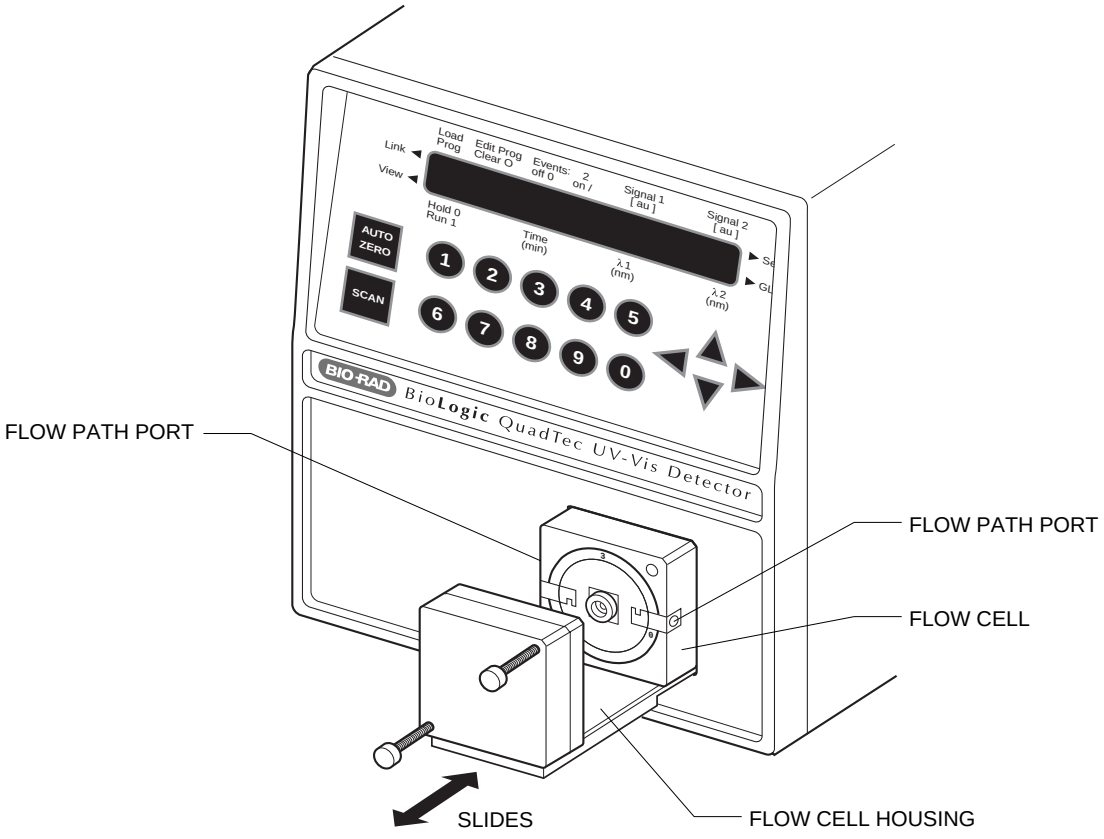


Figure 3-9. QuadTec Detector

The ICM translates the signal from the QuadTec detector and transmits it to the DuoFlow Workstation.

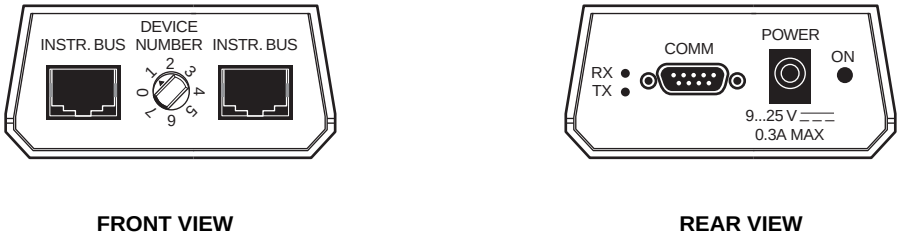


Figure 3-10. Instrument Control Module (ICM)
(front and rear views; for use when the Maximizer is not to be used)

The ICM contains the following:

Front View of ICM Module (required if Maximizer is not in use)

- Address setting: dial should always be set to position 1.
- Instrument bus connector: System Cable 17 connects to the Workstation and the BioFrac fraction collector (if used). (Refer to Figures 3-4 and 3-5.)

Rear View

- Serial Comm connector: System Cable 25 (QuadTec RS232 cable), connects to the QuadTec detector.
- Power connector: System Cable 26, connects to the DuoFlow Workstation DC outlet.

The QuadTec detector is equipped with a universal power supply, which operates with supply voltages from 90 to 260 Volts AC. A manual setting of the supply voltage is not required.

CAUTION! Make sure to use a properly grounded power outlet and the power cable provided with the system.

To connect the QuadTec to the Workstation via the ICM module,

1. Use System Cable 25 to connect from the RS232 connector on the back of the QuadTec to the COMM connector on the ICM module.
2. Use System Cable 26, the ICM power cord, to connect to the back of the Workstation.
3. Use System Cable 17 to connect between an instrument bus connector on the ICM and an available instrument bus connector on the Workstation.
4. If you are using a Bio-Rad device with an instrument bus connector, such as the BioFrac fraction collector, use another System Cable 17 to connect between the instrument bus connectors on the BioFrac and the ICM.

3.6.3 pH Monitor

The pH monitor, available as an option from Bio-Rad, (refer to section 2.5.4 for more information) may be connected to the DuoFlow system in one of two ways:

- To a Workstation connect the Signal Import Module (SIM) included with the pH Monitor. The SIM connects to the DuoFlow Workstation through the bus communication cables (System Cables 17, 18, 19, 21, or 30).
- To a Maximizer, if in use, connect to the rear BNC connector labeled pH.

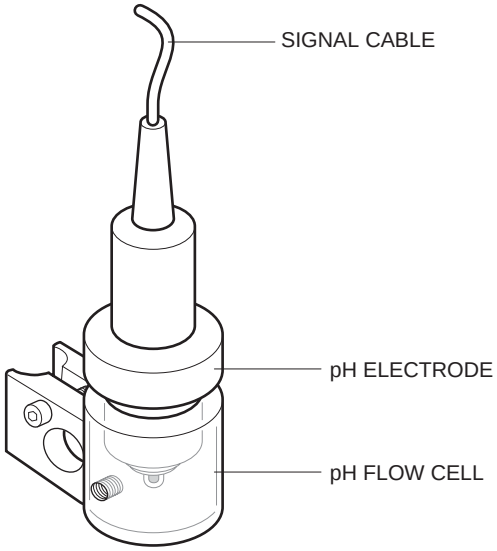


Figure 3-11. pH Monitor

To connect the Bio-Rad pH electrode to the SIM,

1. Connect the output cable from the pH electrode to the connector labeled pH Connector on the SIM.
2. Connect the SIM to the USB Bitbus Communicator by using an Instrument Bus cable (System Cables 17, 18, 19, 21, or 30), as shown below.
3. Connect an external power source to the USB Bitbus device (see Section 2.1.2).

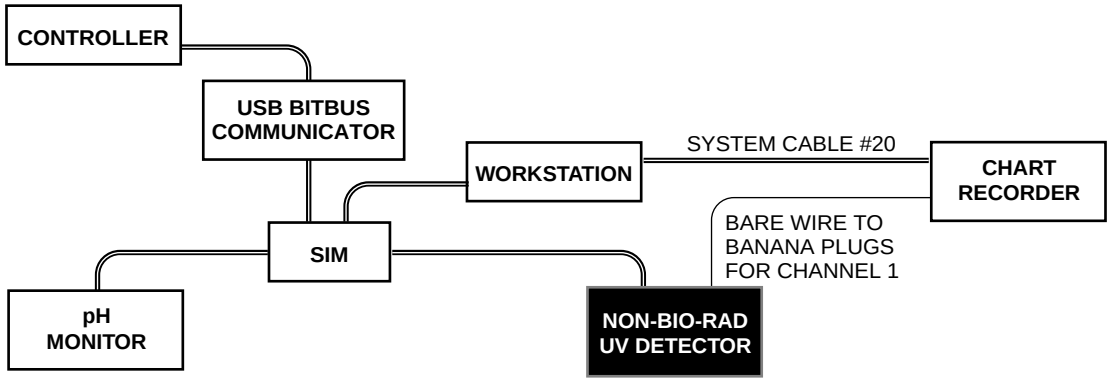


Figure 3-12. SIM Connections

3.6.4 Non-Bio-Rad Detectors

The Signal Import Module (SIM), available from Bio-Rad, allows you to connect a variety of additional devices such as UV, fluorescence, and RI detectors. Before connecting the detector to the SIM, consult the documentation provided with the detector to determine the cable requirements for connecting to the 3-pin connector (+, -, Gnd) on the SIM.

The SIM connects to the DuoFlow Workstation and Controller through the use of the Instrument Bus cables (System Cables 17, 18, 19, 21, or 30), as shown above. Channel 1 signals are sent to the chart recorder by connecting the UV detector to the chart recorder using bare wires to banana plugs. System Cable 20 transmits start/stop, pen up/down, and event mark signals.

3.7 VALVE CONNECTIONS

The DuoFlow system is shipped with an AVR7-3 inject valve. The connection of all valves is similar, as discussed below:

- To connect either the AVR7-3 inject valve or an AVR9-8 stream select valve, mount the valve to a vertical bar on the system rack and connect its cable to any of the connectors labeled Automated Valves 10, 11, or 12 on the rear of the Maximizer, if available. Otherwise, connect it to connectors 4, 5, or 6 on the Workstation.
- To connect an SVT3-2 diverter valve or the SV5-4 buffer select valve, mount the valve to a vertical bar on the system rack and then connect its cable to any of the connectors labeled Solenoid Valves 7, 8, or 9 on the rear of the Maximizer, if available. Otherwise, connect it to connectors 1, 2, or 3 on the Workstation.
- Refer to section 2.6 for more detailed information about each valve.

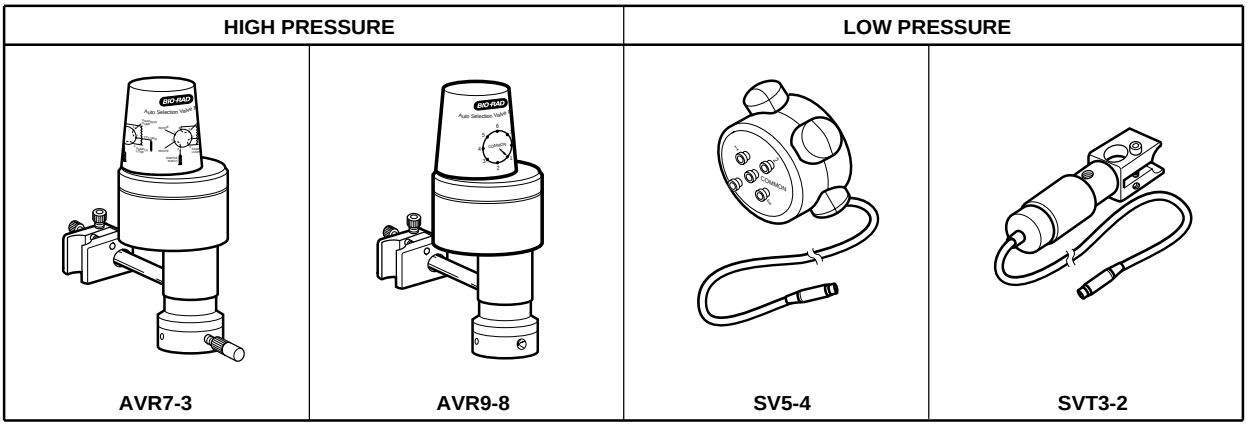


Figure 3-13. DuoFlow Valves

3.8 FRACTION COLLECTOR CONNECTIONS

This section discusses the connections for the following instruments and devices:

- BioFrac fraction collector
- Model 2110 fraction collector
- Model 2128 fraction collector

Refer to section 2.7 for more detailed information about the fraction collectors.

3.8.1 BioFrac Fraction Collector

The BioFrac fraction collector is controlled by the DuoFlow software version 4.0 or greater via the Instrument Bus. The BioFrac fraction collector is connected to the system as discussed below:

1. Connect the USB Bitbus to the Controller as discussed in Section 3.2.
2. Use either of the instrument bus connectors on the rear of the fraction collector to connect the BioFrac fraction collector to the Instrument bus. (see Section 3.3)
3. Select BioFrac in the BioLogic Configuration Utility.

3.8.2 Model 2110 Fraction Collector

The Model 2110 fraction collector may be connected to the Workstation, as discussed below:

1. Connect the DB-9 connector on System Cable 5 to the Model 2110 fraction collector.
2. Connect the bare wires to the AUX connector on the Workstation as follows: Black wire to pin 5 and white wire to pin 9.

3.8.3 Model 2128 Fraction Collector

The Model 2128 fraction collector is controlled by the DuoFlow Controller via the Instrument Bus. The Model 2128 fraction collector is connected to the system as discussed below:

1. Connect the USB Bitbus to the Controller as discussed in section 3.2.
2. Use the instrument bus connector on the rear of the fraction collector to connect the Model 2128 fraction collector to the instrument bus. Since the Model 2128 has only one instrument bus connector, it should be the last device "daisy-chained" to the instrument bus.
3. Select Model 2128 in the BioLogic Configuration Utility.

3.9 PUMP CONNECTIONS

This section discusses the connections for the following instruments and devices:

- Model EP-1 Econo pump
- Econo Gradient Pump (EGP)

3.9.1 Model EP-1 Econo Pump

The EP-1 Econo pump is connected to the system as discussed below:

To connect the EP-1 Econo pump with the DuoFlow system, make the following cable connections:

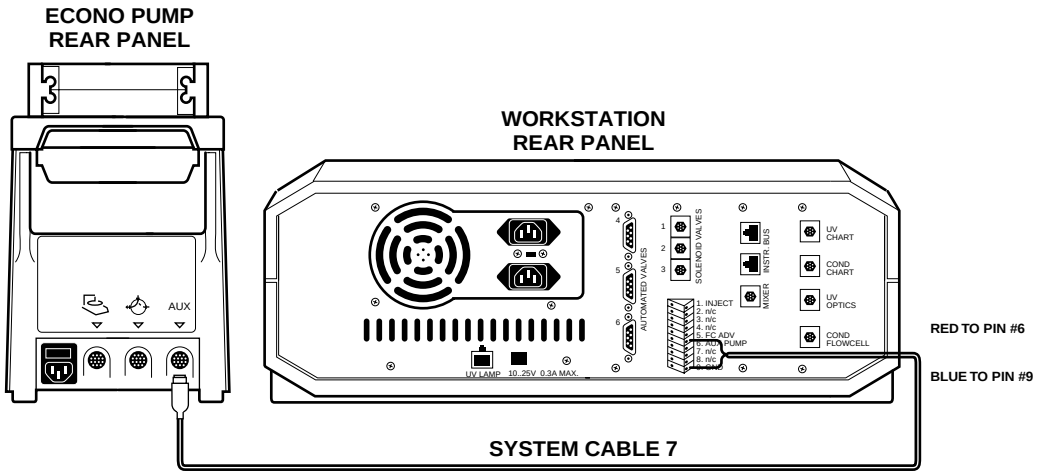


Figure 3-14. Connecting an EP-1 Econo Pump to the BioLogic DuoFlow Workstation

1. On System Cable 7 locate the red and blue wires at the wire end of the cable and connect them to the DuoFlow Workstation.
   Cut short all other wires and insulate with tape.
   a. Connect the red wire to pin 6 of the Aux connector.
   b. Connect the blue wire to pin 9 of the Aux connector.
2. Plug the 8-pin mini-DIN connector into the Aux connector on the rear panel of the Econo pump.

PharMed tubing is recommended for use in the pumphead of the EP-1 Econo pump. For additional information on this pump, consult its user manual. The illustration below shows the Aux pump connected to port 3 of the Inject Valve.

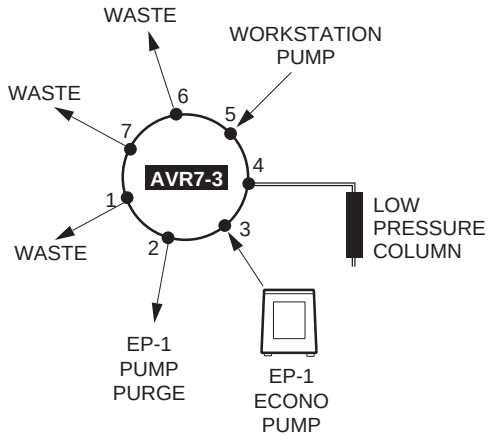


Figure 3-15. Example of Direct Inject Sample Loading using an Econo Pump

The EP-1 Econo pump can be used to load up to 7 samples sequentially when used with an AVR9-8 at the pump's inlet valve, as shown below.

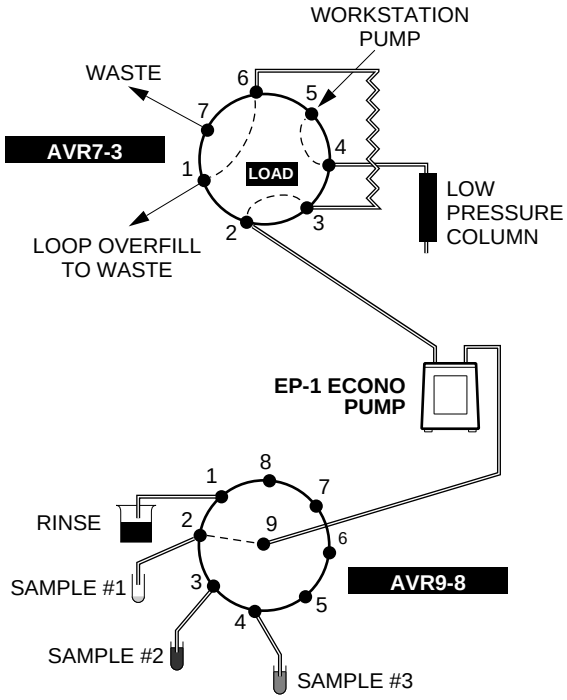


Figure 3-16. Example of Multiple Sample Loading using an Econo Pump

3.9.2 Econo Gradient Pump (EGP)

Refer to section 2.8.3 for more information. The EGP is connected to the Workstation using an Instrument Bus communication cable (System Cables 17, 18, 19, 21, or 30). Connect its power cable to an available outlet. Be sure to use the appropriate fuse. Refer to the separate documentation for the EGP for fuse specifications.

The EGP is connected to the DuoFlow Workstation with Bus communication cable (System cables 17, 18, 19, or 21). When the DuoFlow assumes control of the EGP, the EGP is automatically set to Remote mode.

In Remote mode, the EGP keys provide limited control, allowing only basic observation of EGP operating parameters. For a complete discussion of the EGP, refer to its separate documentation.

The illustration below shows the Aux pump connected to port 3 of the inject valve.

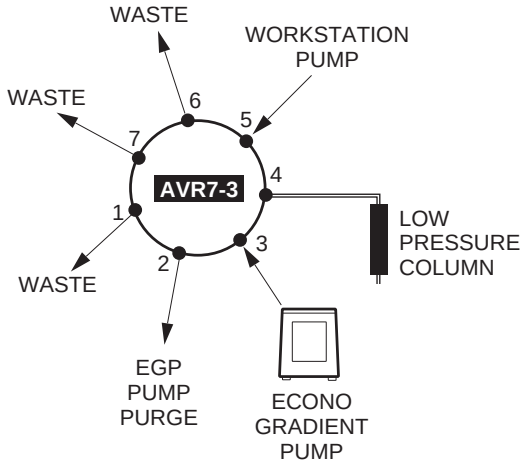


Figure 3-17. Example of Direct Inject Sample Loading using an Econo Gradient Pump (EGP)

The Econo Gradient Pump (EGP) can be used to load up to 7 samples sequentially when used with an AVR9-8 at the pump's inlet valve, as shown below. See Chapter 8 for more details.

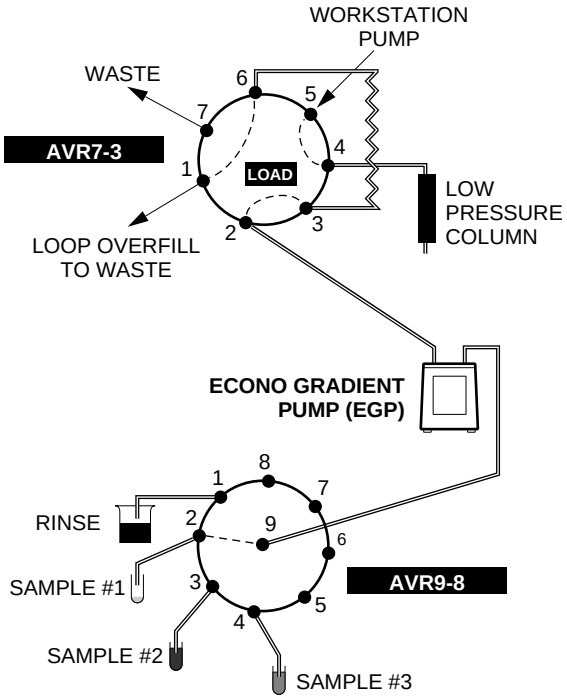


Figure 3-18. Example of Multiple Sample Loading using an Econo Gradient Pump (EGP)

3.10 MODEL 1327 CHART RECORDER CONNECTIONS

The Model 1327 chart recorder is optional. It may be positioned on the rack shelf or on the bench.

1. Connect System Cable 2 between the Workstation and the recorder as follows:
   a. The mini-DIN connector is connected to the connector marked “UV Chart” on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
   b. The DIN connector is connected to the single DIN connector on the side of the chart recorder.
      This cable provides system control of pen up/down, event marks and paper advance, but chart speed MUST be set on the recorder faceplate itself.
2. Conductivity signals are recorded on channel 2 of the recorder using System Cable 4 as follows:
   a. The mini-DIN connector is connected to the connector marked “Cond. Chart” on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
   b. The banana plugs are connected to the connectors marked CH 2 on the side of the recorder (red wire to +, black wire to ground |).
3. Set both channel inputs on the recorder to 1V. Set all other switches to their position marked in green. Connect the power adapter to the chart recorder.

3.11 COMPLETING SYSTEM SETUP

Once you have completed the system setup, turn the units around. Connect the USB cable to the USB Bitbus communicator and connect the instrument bus cables. Plug in the power cords, and turn on the system. In certain laboratory environments, an uninterruptable power supply (UPS) may be required. Bio-Rad offers both 110 V and 220 V UPS configurations; consult your local Bio-Rad representative.

3.11.1 DuoFlow System Network Connections

The DuoFlow system can be connected to an Ethernet network, allowing you to print reports and export files over the network. Refer to the documentation provided with your PC computer for details on cable connection and software setup. Contact your site administrator to ensure proper communication and access with the existing network.

3.11.2 System Power Up

Power up the system. The following is intended as a checklist of instruments and devices that may be connected to the system. The sequence is not important, although it is best to power on the Controller last.

1. Turn on power to the DuoFlow Workstation.
2. Turn on power to the Maximizer, if in use.
3. Turn on power to the following devices and instruments, if they are available:
   a. Power up the QuadTec using the power switch on the rear of the detector.
      The QuadTec detector goes through a startup routine, self-test, and lamp calibration routine.
   b. Power up the auxiliary pump. Auxiliary pumps include the EP-1 Econo pump and the Econo Gradient Pump (EGP).
   c. Power up the fraction collector. Fraction collectors include the BioFrac, the Model 2110, and the Model 2128.
4. Power up the Controller. At this point, the DuoFlow software application may be launched.
5. When the DuoFlow Controller establishes communication with the system, the faceplate in the Manual screen and the status bar at the bottom of the screen show which devices and instruments are connected and communicating with the system. The system is ready for operation.

3.11.3 BioLogic Configuration Utility Software

The BioLogic Configuration utility is used anytime the pumpheads are changed or a Maximizer is installed. It is also used to choose between Bio-Rad's BioFrac and Model 2128 fraction collectors. To run this software:

1. Exit the BioLogic DuoFlow software by selecting Exit from the File drop-down menu.
2. Double-click on the BioLogic Configuration icon.
3. In the BioLogic Configuration Utility window, indicate the pump that will be used with the system, whether or not the Maximizer will be used with the system, and the default Bio-Rad fraction collector (either the BioFrac or the Model 2128).
4. Exit the BioLogic Configuration Utility and double-click on the BioLogic DuoFlow icon to start the software.

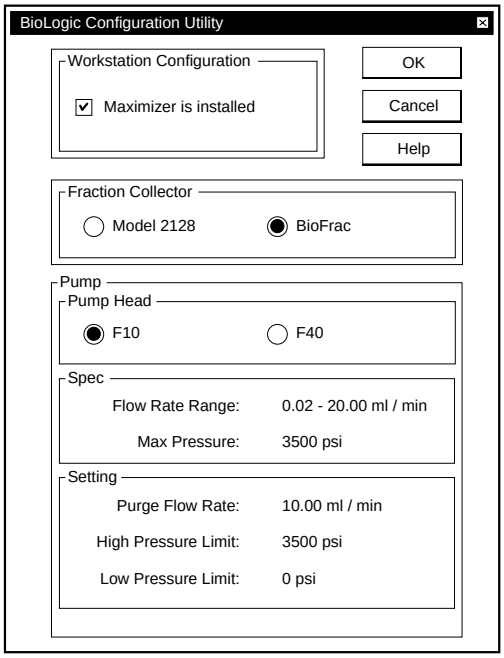


Figure 3-19a. BioLogic Configuration Utility Software Screen

4.0 SYSTEM PLUMBING

This chapter discusses recommended plumbing practices and provides general guidelines for system setup.

The system will work more efficiently if tubing lengths are as short as possible. Bio-Rad provides precut and 1/4-28 fitted, labeled tubing in the Fittings Kit. The illustration below shows where the tubing is designed to be connected. Discussion of how to create your own tubing can be found in the following section.

Use 1/16” (1.6 mm) OD PEEK tubing and 1/4-28 fittings. Use orange PEEK 0.020” (0.51 mm) ID tubing for the F10 pumps, and green PEEK 0.030” (0.76 mm) ID tubing for the F40 pumps. Premade tubing kits are provided with your Fittings kit.

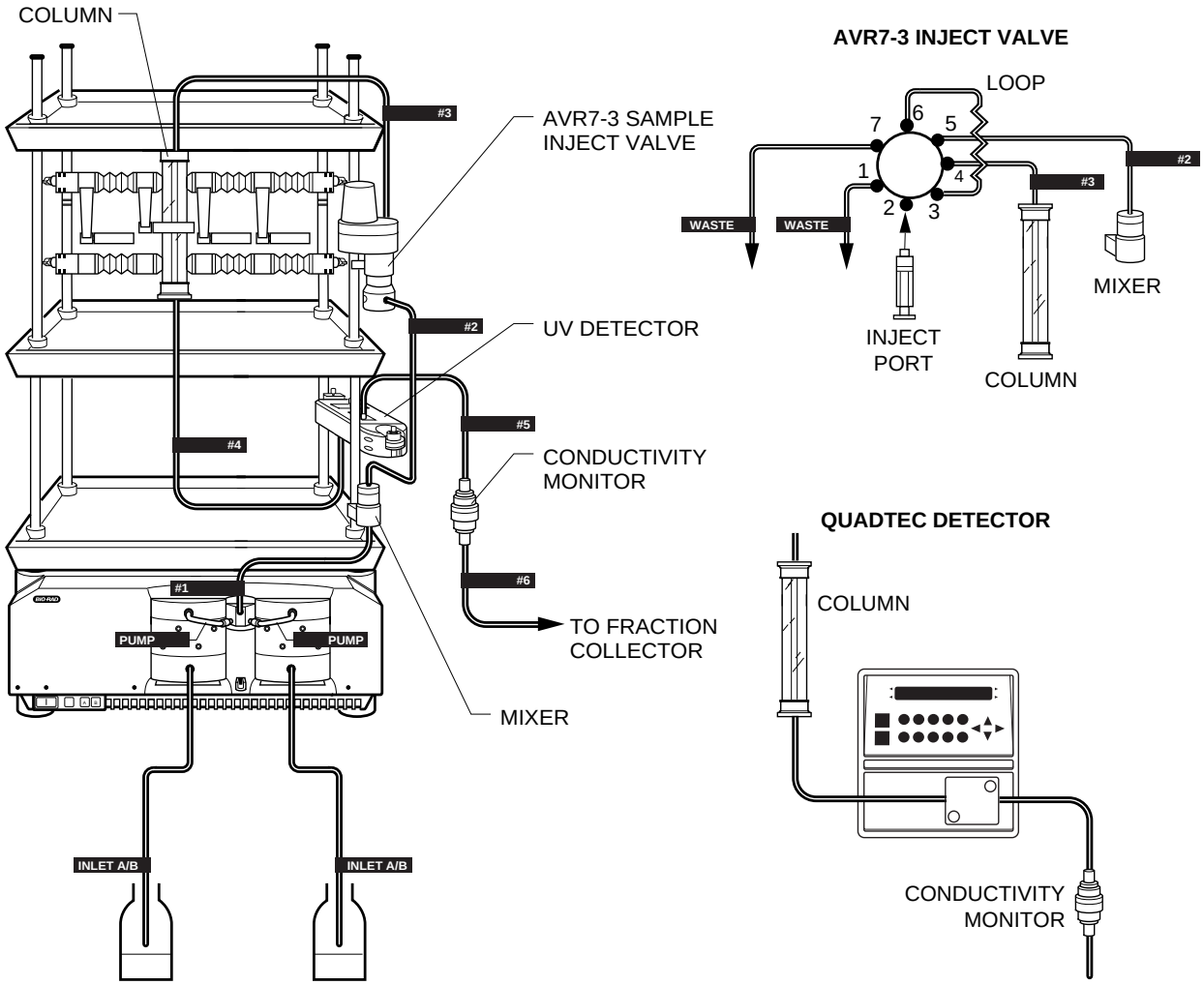


Figure 4-1. System Plumbing using Tubing Kit from Fittings Kit

4.1 GENERAL GUIDELINES FOR CREATING YOUR OWN TUBING CONNECTIONS

The DuoFlow system uses three types of tubing. Use the following table to select the appropriate tubing.

The Fittings Kit comes with fittings, tubing, and an F10 Tubing Kit, with the necessary tubing cut and fitted for each system connection (see Figure 4-1). Complete description of each of these items is provided with the Fittings kit.

Table 4-1.
Tubing Guidelines

Use	Tubing Dimensions	Tubing Material	Fittings	Vol/cm
Pre-pump* (Workstation and Aux pumps)	1/8” OD x 0.062” ID (3.2 mm OD x 1.6 mm ID)	clear, PTFE	Super flangeless fittings for 1/8” (3.2 mm) OD tubing	20 µl
Post-pump**	1/16 OD x 0.02” ID (1.6 mm OD x 0.5 mm ID)	clear, Tefzel	Super flangeless fittings for 1/16” (1.6 mm) OD tubing	2 µl
Post-pump** with F10 pumphead	1/16” OD x 0.020” ID (1.6 mm OD x 0.5 mm ID)	orange, PEEK	1/4-28 Super flangeless fittings for 1/16” (1.6 mm) OD tubing	2 µl
Post-pump** with F40 pumphead	1/16” OD x 0.030” ID (1.6 mm OD x 0.76 mm ID)	green, PEEK	1/4-28 Super flangeless fittings for 1/16” (1.6 mm) OD tubing	4.5 µl

* Pre-pump refers to all tubing leading up to pump inlet port. Pumps include Workstation pumps, EP-1 pump, and Econo Gradient Pump. Maximizer uses pre-pump tubing.
**Post-pump refers to all tubing following the pump outlet port.

When plumbing the system, be sure to keep tubing lengths to a minimum. All fittings should be finger-tight. Do not over-tighten, as you risk damaging the connection.

Ferrule Installation: 1/8” (3.2 mm) OD and 1/16” (1.6 mm) OD tubing

1. Cut the tubing with the tubing cutter provided in the Fittings Kit. This will give a flat, clean cut to the tubing; this is important in making a fitting that does not leak.

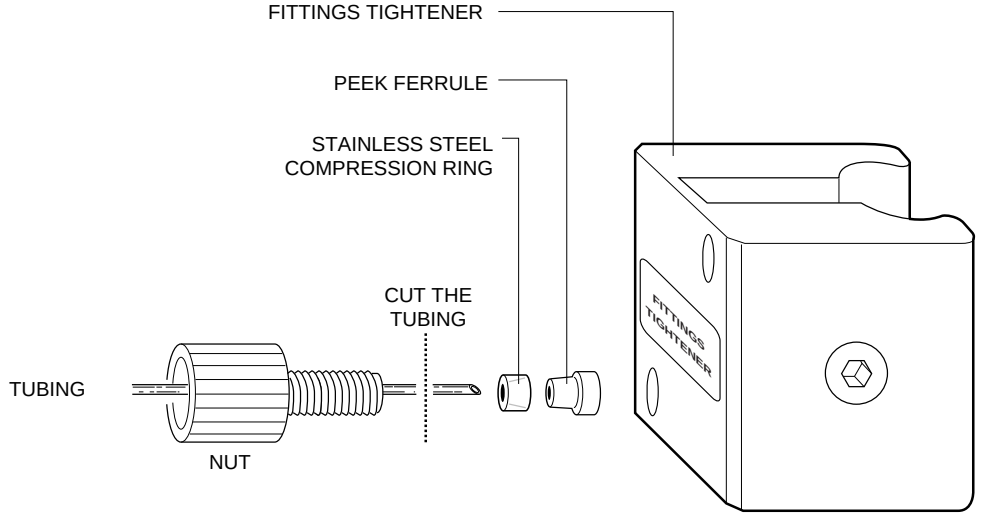


Figure 4-2. Making 1/4-28 flat bottom fittings

2. Slide the nut, stainless steel compression ring, and the ferrule, in that order, onto the tubing as shown in Figure 4-2. The flattened end of the compression ring should face towards the nut with the tapered end facing the tapered end of the brown ferrule.
   The stainless steel lock ring is *not* in the fluid path, so biocompatibility is maintained.
3. Allow the tubing to extend slightly beyond the end of the ferrule.
4. Place the fitting and tubing into the green fittings tightener. Do not allow the tubing to slip out of the ferrule.
5. Tighten with your fingers to seat the ferrule onto the tubing, but do not over-tighten. The ferrule should be flush with the tubing.
6. Once the fitting is made, the compression ring and ferrule should adhere to the end of the tubing while the nut will be moveable.

4.2 PLUMBING A DUOFLOW SYSTEM

This section discusses how to create your own plumbing arrangement of a DuoFlow system. The plumbing for each of the DuoFlow valves is discussed later in the chapter.

The Fittings Kit includes an F10 tubing kit that includes all tubing required for a basic installation. Refer to Figure 4-1.

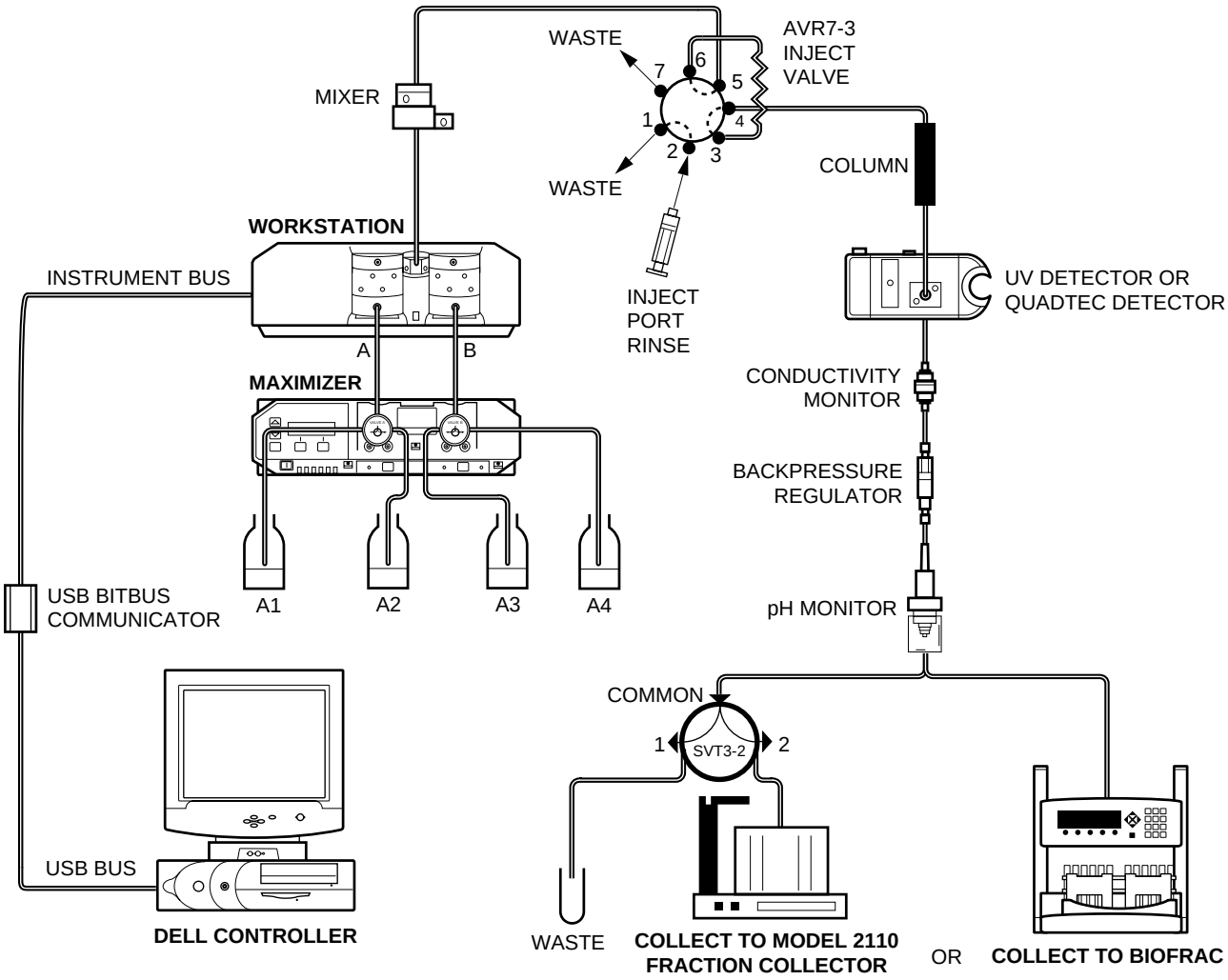


Figure 4-3. System Plumbing with Maximizer

1. Plumbing the Maximizer.

The Maximizer Tubing Kit provides colored FEP PTFE 1/8” OD, 0.062” ID, prefitted, tubing lengths.

Plumbing the Buffer Reservoirs to the Inlets on Maximizer Valves A and B

a. From the Maximizer Tubing Kit, identify the following:

The red tubing labeled Inlet A1 connects the buffer container to the Maximizer valve port A1.

The blue tubing labeled Inlet A2 connects the buffer container to the Maximizer valve port A2.

The yellow tubing labeled Inlet B1 connects the buffer container to the Maximizer valve port B1.

The green tubing labeled Inlet B2 connects the buffer container to the Maximizer valve port B2.

For complete discussion of Maximizer tubing installation, refer to the Maximizer Tubing Kit diagram.

b. Screw the tubing into the inlet connectors on the sides of the valves. Ensure a firm connection but do not over-tighten.

Plumbing the Maximizer Valve Outlets to the Workstation Pump Inlets

c. Connect the two preformed fittings provided between the Maximizer valve ports and the Workstation pump inlet ports. Connect to the Workstation first.

d. Because the tubing is rigid, you will need to lower the Maximizer valves in order to connect the tubing. This requires loosening the two screws at the base of each valve so that you can tilt the valve downward. Refer to the illustration below.

e. Insert the tubing fitting into the Maximizer outlet port and screw in the fitting.

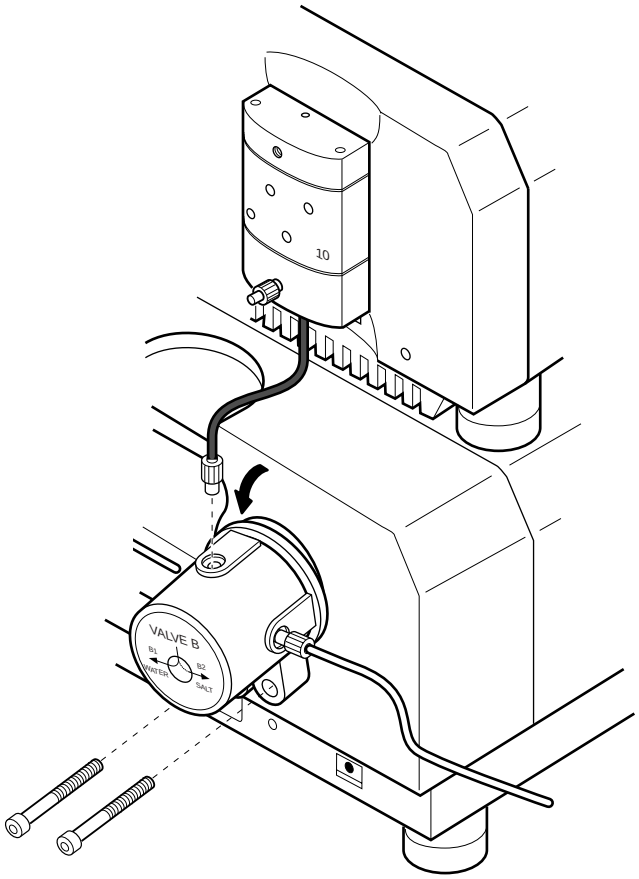


Figure 4-4. Maximizer Plumbing

2. Plumbing the Workstation pump Inlets.

a. Workstation pump inlets, attach two fittings to 1/8” (3.2 mm) OD PTFE tubing as described earlier. (See Section 4.1, General Guidelines for Creating Your Own Tubing.)

To connect the SV5-4 buffer select valve or the SVT3-2 valve before the pump inlet, 1/8” (3.2 mm) OD PTFE tubing is used.

b. Screw the tubing into the inlet connectors on the bottom of the Workstation pump housing. Ensure a firm connection but do not over-tighten.

c. Place opposite end of tubing in solution bottles A and B. Refer to figure 4-1. If an SV5-4 valve is in use, attach the pump inlet tube to its common port. Refer to section 2.6.3.

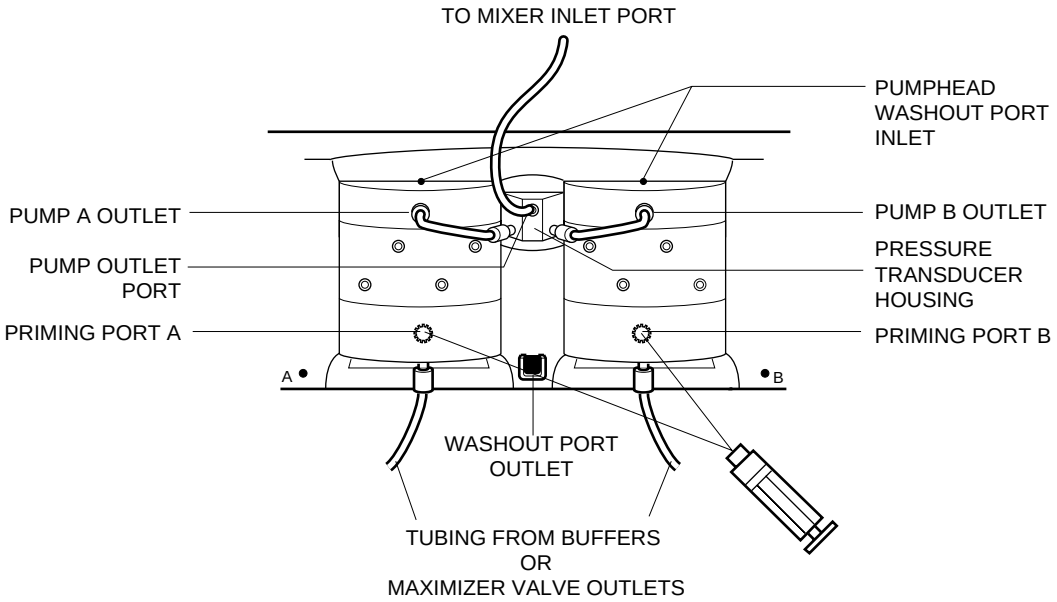


Figure 4-5. Plumbing Connections to the Workstation Pump

3. Plumbing from the Workstation pumpheads to the Transducer (Figure 4-3).

a. Use the two pieces of tubing labeled PUMP from the tubing kit or make two fittings using 1/16” (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings, as described in section 4.1.

b. Connect the tubing to each pumphead outlet port and to each transducer inlet port. Ensure a firm connection but do not over-tighten.

4. Plumbing from the Transducer to the Mixer (Figures 4-2 and 4-3).

a. Use the tubing labeled #1 from the tubing kit or make a fitting using 1/16” (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings.

b. Connect the tubing to the transducer outlet port and to either of the inlet ports on the mixer. Plug the unused mixer inlet port using the plug provided for this purpose. Ensure a firm connection but do not over-tighten.

5. Plumbing from the Mixer to the AVR7-3 Inject Valve.

a. Use the tubing labeled #2 from the tubing kit or cut a suitable length of 1/16” (1.6 mm) OD orange PEEK tubing that will reach from the mixer outlet port to port 5 of the inject valve. Attach 1/4-28 fittings to each end.

b. Connect the tubing to the mixer outlet port and to port 5 on the inject valve. Ensure a firm connection but do not over-tighten.

6. Plumbing the AVR7-3 Inject Valve.

a. The inject port assembly and needle are included with each AVR7-3 inject valve. Screw this assembly into port 2 of the valve until it is secure.

b. Connect the sample loop to ports 3 and 6.

c. Plumb the inject valve according to Figure 4-6. Use the tubes labeled "waste" or make fittings using 1/16” (1.6 mm) OD Tefzel tubing and 1/4-28 fittings for waste lines 1 and 7.

d. Use the tube labeled #3 or make a fitting using 1/16" (1.6 mm) OD Tefzel tubing and 1/4-28 fitting to go from port 4 to the column.

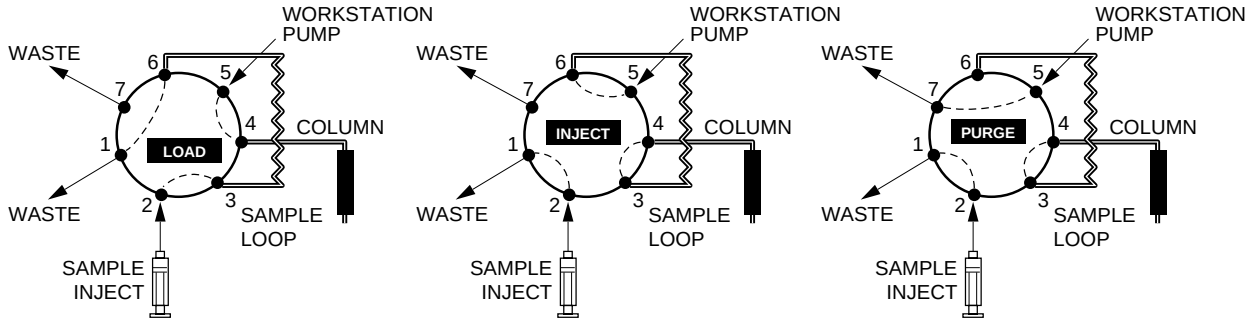


Figure 4-6. Inject Valve Plumbing for an AVR7-3

7. Plumbing the UV Detector and Conductivity Monitor.

a. Connect a piece of 1/16” (1.6 mm) OD orange PEEK tubing (tube #4 in the tubing kit) between the column outlet and the bottom inlet of the UV detector flow cell using 1/4-28 fittings. If you are using the QuadTec detector, connect it to the flow cell which is bidirectional, using 10-32 fittings provided with the QuadTec.

b. Use the tube labeled #5 in the tubing kit or cCut approximately 8 cm of 1/16” (1.6 mm) OD orange PEEK tubing and attach 1/4-28 fittings. Connect one end into the top of the UV detector flow cell. Connect the other end into the Conductivity monitor flow cell which is bi-directional. If you are using a QuadTec detector, connect tubing from its flow cell outlet using a 10-32 fitting to either port of the Conductivity monitor using 1/4-28 fittings.

c. Place the Conductivity flow cell into the notch of the optics bench. This is a gentle push-fit. There is a tag (with a number) attached to the conductivity cable. This number is the flow cell constant and must be entered in the software before beginning a run. Refer to Table 5-6, page 5-10 Utilities drop-down Menu: Conductivity Flow Cell Constant Calibration.

d. For flow rates below 10 ml/min, insert the 40 psi backpressure regulator after the conductivity monitor. Plumb the backpressure regulator following the direction of the arrow.

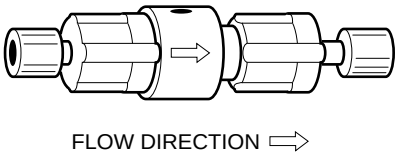


Figure 4-7. Backpressure Regulator

The backpressure regulator is required to help eliminate bubbles from becoming trapped in the detector flow cell.

When using low pressure columns such as an Econo-Pac® cartridge or Econo column, plumb the 40 psi backpressure regulator at the Workstation pump outlet. This aids in seating the check valves, preventing permanent damage to the cartridge or column.

Refer to section 2.9.5, for more information.

8. Connection to a Fraction Collector.

Connection to a BioFrac Fraction Collector

a. Use the tube labeled #6 in the fittings kit or make a fitting using 1/16” (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings.

b. Connect the fitting between the Conductivity flow cell (or the backpressure regulator or pH monitor flow cell, if they are installed) and the BioFrac. (For complete instructions, refer to the documentation for the BioFrac fraction collector.)

Connection to a Model 2128 Fraction Collector

a. Use the tube labeled #6 in the fittings kit or make a fitting using 1/16” (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings.

Assure that the tubing length allows unrestricted movement of the fraction collector arm.

b. Connect the fitting between the Conductivity flow cell (or the backpressure regulator or pH monitor flow cell, if they are installed) and the drop-head of the Model 2128 or the Model 2128 diverter valve. (For complete instructions, refer to the documentation for the Model 2128 fraction collector.)

Connection to a Model 2110 Fraction Collector and a SVT3-2 Fraction Collector Diverter valve.

a. Connect a piece of 1/16” OD orange PEEK tubing between the Conductivity flow cell (or the backpressure regulator, if that was installed) and the SVT3-2 valve common port (labeled “**Common**”) using the 1/4-28 fittings.

b. Connect a piece of 1/16” OD Tefzel tubing from port 1 of the SVT3-2 valve (see Figure 4-3) for use as a waste line.

c. Connect a piece of 1/16” OD Tefzel tubing from port 2 of the SVT3-2 valve (see Figure 4-3) to the Model 2110 fraction collector drop-head. This tubing sits inside the drop-head, and no fittings are required.

4.3 PRIMING THE SYSTEM

Before a method can be run, the system must filled with buffer and all air bubbles purged from the system. The following procedure should be used to prime the system for the first time and when changing buffers.

1. **Priming the Workstation pump.**
   a. Immerse the Workstation pump A and B or Maximizer A1, A2, B1 and B2 inlet lines into a container of HPLC grade (filtered, degassed) or other high quality water.
   b. Place the 10 ml luer syringe (supplied with the fittings kit) in the priming port of pumphead A. If a Maximizer is connected, select Inlet A1.
   c. Turn the priming port counter-clockwise to open the port and gently draw water into the syringe from the pumphead.
   d. Repeat this operation until no air bubbles are visible in the inlet tubing.
   e. Disconnect the pumphead outlet tube and hold a beaker up to the port. With the syringe full of water inject water into the priming port using several short pulses to dislodge any trapped air bubbles. Once all the bubbles have been dislodge, close priming port and reconnect the pumphead outlet tube.
   f. Repeat this priming procedure for the pump B inlet or inlets A2, B1 and B2, if a Maximizer is connected.

2. **Flush the System Through to the Fraction Collector.**
   a. Take the column out of line if it has been connected.
   b. From the Manual screen, place the AVR7-3 injection valve into the Purge position. The AVR7-3 valve control panel is labeled with the valve type and the Workstation port where the valve is connected. For example, if the valve is connected at Workstation port 4, the panel will be named "AVR7-3 at port 4." The AVR7-3 valve the panel shows three valve positions Load (L), Inject (I) and Purge (P).
   c. Press the Purge buttons A and B on the front of the Workstation. The Workstation pump will run and the indicator lights will flash green. The default purge flow rate is 10 ml/min for F10 pumps and 40 ml/min for F40 pumps. The default purge flow rate can be changed from the Options/Manual Setup menu option on the Manual screen.
   d. Run both pumps for about 2 minutes. If a Maximizer is connected to the system, place the valve inlets at position A2 and B2 for about a minute and then at A1 and B1 for an additional minute. Press the Purge buttons again to stop the pump.
   e. From the Manual screen place the AVR7-3 injection valve into the Inject (I) position.
   f. Set the pump flow rate to 1.0 ml/min and start the pump. Water will now flow through the sample loop, the UV Conductivity flow cells, and ultimately to the fraction collector.
   g. After the system is filled, change the AVR7-3 injection valve to Load and monitor the pressure displayed on the status bar for a few minutes. If the pressure variation exceeds ± 10 % repeat step 1e until the pressure is stable. It is best to monitor the pressure with some backpressure on the pumps (such as the 40 psi backpressure device). See Section 11.3 for additional information.

5.0 INTRODUCTION TO THE SYSTEM SOFTWARE

The BioLogic DuoFlow system software is run on computers running the Microsoft Windows® 2000 operating system. This chapter discusses the DuoFlow system software version 5.0.

5.1 SYSTEM INTERFACE

The Manual screen (Figure 5-1) is the first screen displayed when the BioLogic software is started. This screen, like all DuoFlow screens, is grouped into the system menus, the control window, and the status bar. The use of the Control window functions is discussed in Chapters 6 and 7 and in the separate documentation for the Econo Gradient Pump (EGP) and the QuadTec UV/VIS detector. **Note that many menus and tool bar functions are grayed out and inacessible until you select, or enter a User name in the Browser, see chapter 6.**

- Browser screen: Chapter 6
- Manual screen: Chapter 7, section 7.1
- Setup screen: Chapter 7, section 7.2
- Protocol screen: Chapter 7, section 7.3
- Run screen: Chapter 7, section 7.4
- Post Run screen: Chapter 7, section 7.5

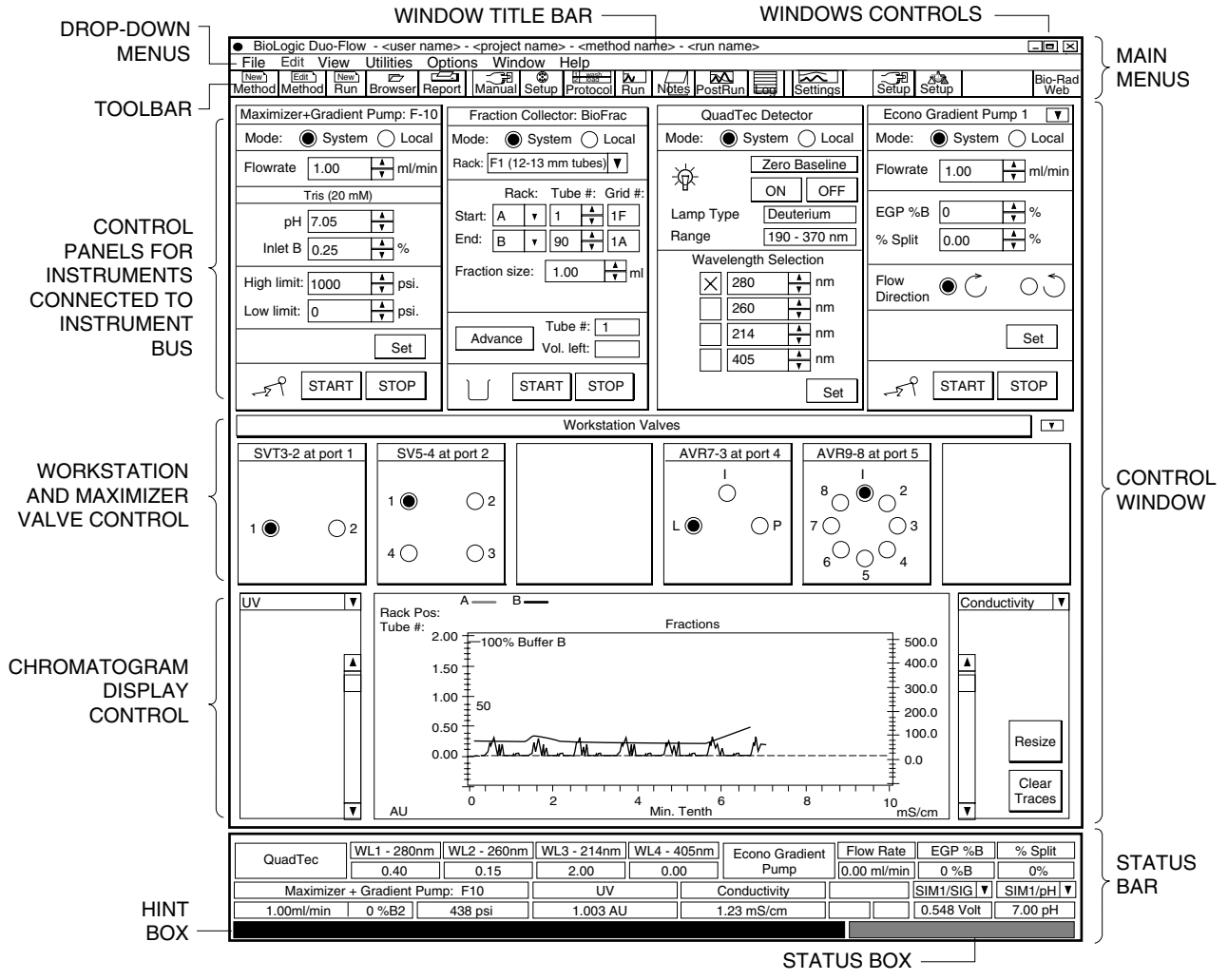


Figure 5-1. Layout of the Screen Display, showing the Manual Screen

The DuoFlow system is controlled and monitored using the following:

- **Toolbar:** The toolbar is the primary navigation tool for the system software.
- **Drop-down menus:** The drop-down menus provide access to advanced functions. Some functions found on the toolbar are duplicated in the drop-down menus.
- **Status bar**: The status bar provides realtime information about active instruments and devices connected to the system, for example system backpressure and detector AU readings.

Items shown in gray are not currently active.

5.2 STANDARD MOUSE AND KEYBOARD FUNCTIONS

The DuoFlow system is supplied with a Dell PC computer. The left mouse button is used with system software, except as noted.

Table 5-1. Special Function Keys

Special Function Keys	Description
F2	**Hold until Keypress:** Used during run to continue a method (i.e., satisfy the Hold) when the method includes a “Hold until Keypress” step.
F1	**Help:** Displays the Help menu for the currently displayed screen.
Esc	**Esc:** Functions as an alternative to the Cancel button in a dialog box.
Alt	**Alt:** Some system commands can be executed either by selecting them from a drop-down menu or by holding down the **Alt** key and then pressing the appropriate character key.

5.3 SYSTEM MENUS

The system menus consist of both drop-down menus and the toolbar. In some cases, identical functions are found in both areas. Advanced features are located only in the system drop-down menu.

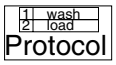
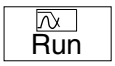
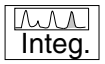
5.3.1 Toolbar Buttons

The function of each toolbar button defined in Table 5-2 is duplicated in the File and View drop-down menus..

Table 5-2.
Toolbar Buttons

Button	Description
New Method	Opens the New Method dialog used to create new methods or load method templates. The new method is saved into the selected User and Project.
Edit Method	Copies the currently open method, renames it and places it in the Protocol Editor for editing. The new method is assigned a default name (<method name>. <version number>) that can be changed by the user.
New Run	Creates and names a new run for the current method and opens the Run screen. Pressing Start in the Run screen starts the run.
Browser	Opens the Browser and displays all the users, projects, methods and runs associated with the database. Many of the menu and toolbar options will remain grayed out until a user name is selected. See Chapter 6 for more detail on the Browser screen.
Report	Opens the Print Report dialog that is used to specify the type of run reports to print.
Manual	Opens the Manual screen that is used for manual control and monitoring of the devices connected to the Workstation and instrument bus.
Setup	Opens the Device Setup screen that is used to specify the devices required for the current method. The Device Setup screen is used to name the pump inlet ports; add valves and name the valve positions; add detectors; add a fraction collector; add auxiliary pumps; and add Buffer Blending (Maximizer systems only) for the method. This screen is automatically opened after the New Method button is selected.

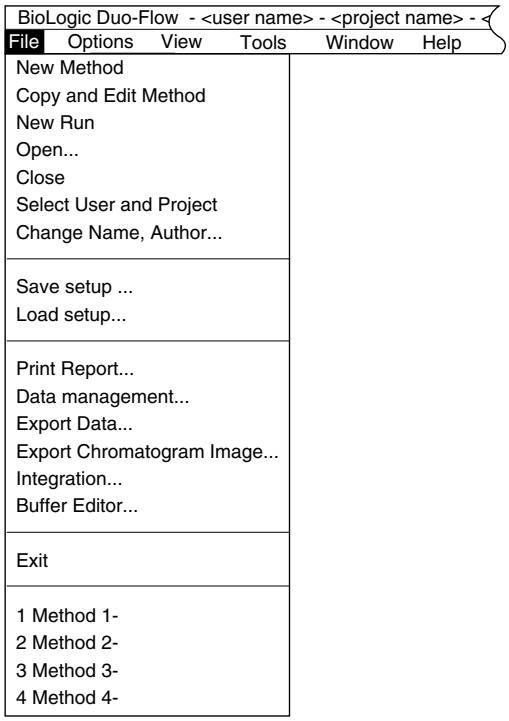
Table 5-2. Toolbar Buttons (continued)

Button	Description
	Opens the Protocol screen for creating and editing a method.
	Opens the Run screen, from which you can start a run of the currently open method. You will be prompted for a new run name prior to launching the run.
	Opens the Notes screen that allows you to enter additional information about your method and/or run. Notes are always editable, even after a run is executed.
	Opens the PostRun screen that allows you to view and customize your chromatogram prior to printing.
	Opens the run log (Read Only) for the current run and displays the events that occurred for the run. The button for the EZLogic integration program replaces this button when it is installed.
	EZLogic Integration button appears when the EZLogic integration software is installed; this button replaces the Log button.
	Enables the selection of up to 8 instrument traces to display on the screen and on the printed report. The trace options are: UV detector, Conductivity monitor, pH, system backpressure, theoretical %B concentration, four QuadTec wavelengths, and detector traces acquired via the System Interface Module (SIM).
	Available only in the Manual screen. Displays a screen that enables the following inputs: • Econo Gradient Pump (EGP) split time period when the EGP is used. • QuadTec UV/VIS detector time constant when the QuadTec detector is used. • Pump purge rate. • Signal Import Module (SIM) signal parameters.
	Available only when the Maximizer is used with the system. This allows you to switch between buffer blending and non-buffer blending modes. In buffer blending mode, a window listing buffer systems available for use is displayed.
	If your controller has internet access, selecting this button takes you to the Bio-Rad web page.

5.3.2 Drop-down Menus

The drop-down menus are discussed in the following tables. **Many menus and tool bar functions will be grayed out and inacessible until you select, or enter a User name in the Browser, refer to chapter 6.**

Table 5-3. File Drop-down Menu



The File menu consists of the following:

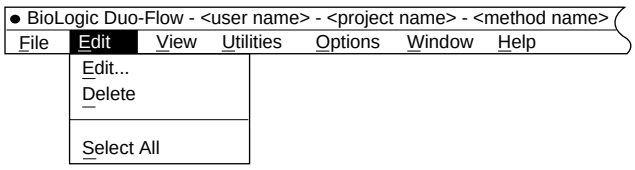
- **New Method**: Opens the New Method dialog used to create new methods or load method templates. The new method is saved into the selected User and Project.
- **Copy and Edit Method**: Allows you to use an existing method as a template for a new method. A copy of the method is made, and you will be prompted for a new method name or the methods will automatically be numbered consecutively.
- **New Run**: Creates and names a new run for the current method and opens the Run screen. Pressing Start in the Run screen starts the run.
- **Open**: Opens the Browser screen. Refer to Chapter 6, Browser Screen.
- **Close**: Closes the current method and/or run.
- **Select User and Project...**: Allows you to select the user and project for the system.
- **Change Name, Author...**: Allows you to change the method/run name, author, and description. This function is inactive once a run has been run.
- **Save setup...**: Allows you to save and name different instrument setup configurations in the Setup screen and to set one as the default.
- **Load setup...**: Recalls and loads a saved setup.

Table 5-3. File Drop-down Menu (continued)

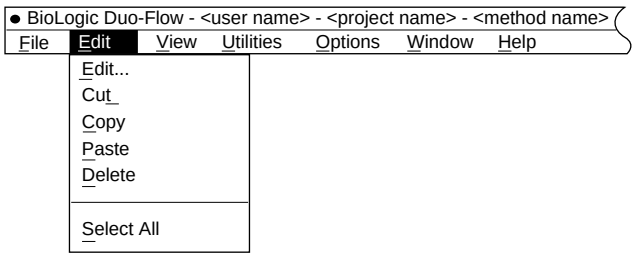
- **Print Report**: Allows you to print a report for the currently open method, including its setup and run data, run results, and the run log report.
- **Data Management**: Displays the Browser screen, from which you can copy and move run data. Refer to Chapter 6, Browser Screen.
- **Export Data**: This feature is used to set data export parameters and export data text files. This feature is available from the PostRun screen.
- **Export Chromatogram Image**: This feature exports a chromatogram image in a Windows Meta File (.WMF) format. This feature is available in the PostRun screen.
- **Integration:** Launches the Bio-Rad EZLogic integration software. Contact Bio-Rad Technical Support at 1-800-4-BIORAD in the USA or your local Bio-Rad representative for more information on EZLogic.
- **Buffer Editor:** Opens the Buffer Editor that is used to create new Buffer Blending buffer systems.
- **Exit**: Exits the BioLogic system software and returns to the Windows desktop.
- **1 through 4**: Display the last four methods and runs used.

Table 5-4. Edit Drop-down Menu

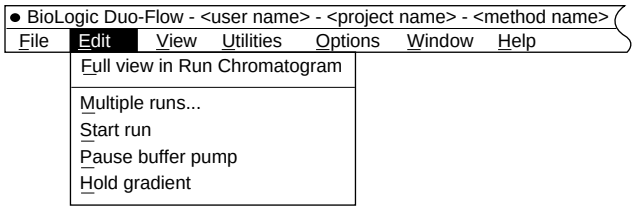
Edit menu: Setup Screen



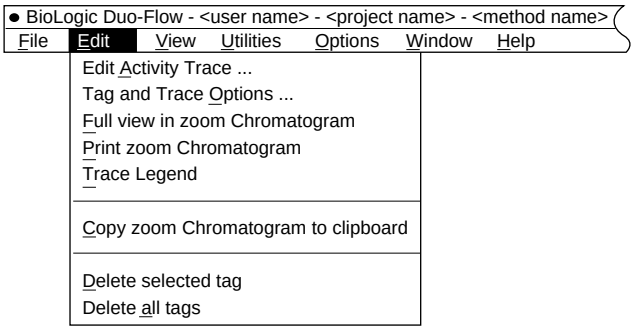
Edit menu: Protocol Screen



Edit menu: Run Screen



Edit menu: Post Run Screen



Note: The Edit menu is not available in the Manual or Browser screens.

The contents of the Edit menu depends upon the displayed screen as indicated above. In most instances, the item in the drop-down menu also appears in the system toolbar; exceptions are noted below.

Setup Screen

- **Edit...**: Allows you to edit the selected device in Setup.
- **Delete**: Deletes the currently highlighted device in Setup. To delete all devices, first select Select All, as described below.
- **Select All**: Highlights all devices in Setup. (Not available from the toolbar.)

Protocol Screen

- **Edit...**: Displays the Edit window for the step selected in the protocol.
- **Cut**: Cuts (deletes) the currently highlighted step from the protocol. A cut step may be pasted elsewhere.
- **Copy**: Copies the currently highlighted step so that it can be pasted elsewhere in the protocol.
- **Paste**: Pastes the cut or copied step into the protocol.
- **Delete**: Deletes the currently highlighted step from the protocol.
- **Select All**: Highlights all protocol steps. Cut, Copy, and Delete then act on all steps. To remove the highlighting from individual steps, hold down the Ctrl key and click the mouse over the desired step. (Not available from the toolbar.)

Run Screen

- **Full view in Run Chromatogram**: Zooms out to show the full view for the run.
- **Multiple runs...**: Specifies the number of times the method is to run.

Table 5-4. Edit Drop-down Menu (continued)

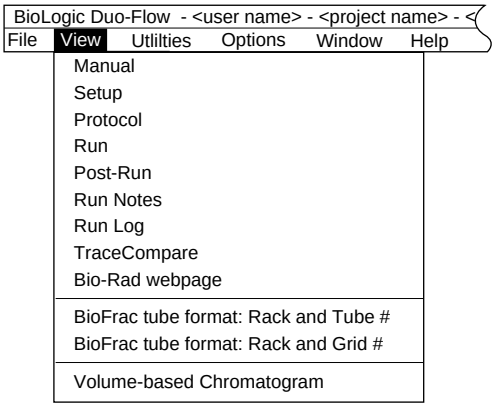
Run Screen (continued)

- **Start run**: Starts the run.
- **Pause buffer pump**: Pauses progression of method's protocol. Stops gradient pumps and the method time (volume) does not advance. From Pause, you can Abort, Continue, or Edit-During-Run.
- **Hold gradient**: Holds the current %B gradient pump conditions and halts the advance of the method's protocol (including fraction collection). The method time (volume) does not advance. From Hold, you can Abort, Pause, or Continue.

Post Run Screen

- **Edit Activity Trace...**: Allows you to input post run sample activity data obtained off-line, refer to Section 7.5.4.
- **Tag and Trace Options...**: Displays the Post Run Tags window that allows you to specify which traces are to be displayed, and name peaks. Also available from the **Tag** button in the Toolbar, refer to Section 7.5.3.
- **Full View in zoom chromatogram**: Allows you to view the complete chromatogram. Also available from the **Full View** button in the system toolbar.
- **Print zoom chromatogram**: Allows you to print the complete chromatogram. Also available from the **Print** button in the toolbar.
- **Copy zoom chromatogram to clipboard**: Copies to clipboard the complete chromatogram. From the clipboard, it can then be copied into other applications. Not available from the toolbar.
- **Delete selected tag**: To delete a tag, highlight the selected tag before using this function. Also available from the **Del. Tag** button in the toolbar.
- **Delete all tags**: Deletes all tags in the chromatogram. Not available from the toolbar.
- **Trace Legend**: Displays different line formats for each of the chromatogram traces. This is useful for distinguishing traces when printing to a black and white printer.

Table 5-5. View Drop-down Menu

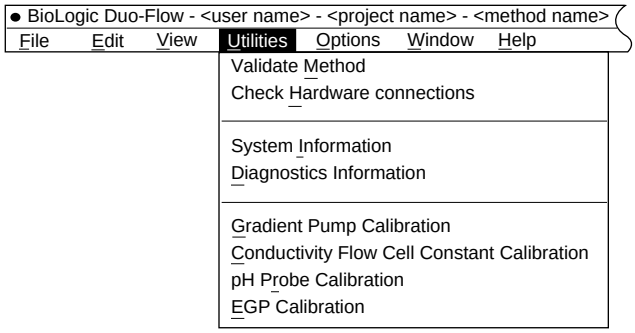


The contents of View menu remain the same in each displayed screen.

- **Manual**: Displays the Manual screen for individual control of installed instruments and devices in the system.
- **Setup**: Displays the Setup screen, which allows you to specify the components required for your method.
- **Protocol**: Displays the Protocol screen for creating and editing chromatography steps in the method.
- **Run**: Displays the Run screen, from which you can initiate a sample run of the open method.
- **Post Run**: Displays the Post Run screen from which you can view the chromatogram, and apply tags to UV, Conductivity, and/or %B traces.
- **Run Notes**: Displays the run notes screen used to store information such as sample description, column type, operator, buffer(s), flow rates, gradients, chart speed, fraction size, and general notes. These notes are printed with the report.
- **Run Log**: Displays a log of all events occurring during the run. This information cannot be edited. The Run Log may be disabled by selecting Edit User Preferences from the Option drop-down menu. It is recommended, however, that the Run Log be active for assistance in troubleshooting.

  Note : If the Bio-Rad EzLogic Integration software option is installed, the Log toolbar button is replaced by the Integ toolbar button. To display the Log window, select Run Log from the View drop-down menu.
- **TraceCompare**: Allows you to compare the trace results from different runs.
- **Bio-Rad webpage**: If your Controller has internet access, you can use this button to access the Bio-Rad web page.
- **BioFrac tube format**: **Rack and Tube #/Rack and Grid #**: Toggles the chromatogram fraction collection trace between "Rack and Tube#" and "Rack and Grid#" mode (see Figures 7-5a, 7-5b, 7-11a and 7-11b for examples).
- **Volume Based Chromatogram/Time Based Chromatogram:** Toggles the chromatogram horizontal axis between time and volume mode (see Figures 7-5a, 7-5b, 7-11a and 7-11b for examples).

Table 5-6. Utilities Drop-down Menu

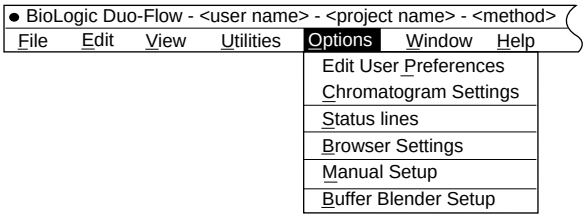


The Utilities menu selections relate to system options, and remain the same in each displayed screen. The Utilities menu consists of the following:

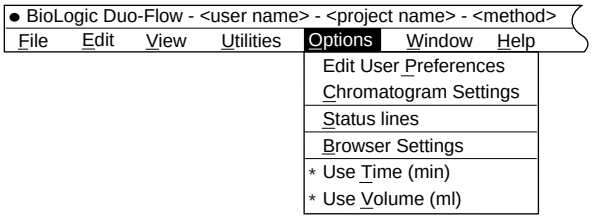
- **Validate Method**: Verifies that the devices required by the method Protocol have been defined or selected in the Setup screen. Validation is automatically run at the start of a new run.
- **Check Hardware connections**: Checks that all devices listed in the Setup screen are electrically connected to the system. The mixer is always assumed to be connected, so it does not appear in the Setup screen and its connection is not checked.
- **System Information**: Displays the current system configuration, including each instrument in the system and its firmware version number, the Windows® version number, available hard disk space, and the number of methods and runs in the database.
- **Diagnostics Information**: Displays information for service diagnostic purposes only.
- **Gradient Pump Calibration**: Available only from the Manual screen, and it is typically used only after servicing of the pump. It allows the user to calibrate the gradient pump flow rate and zero the system pressure gauge.
  Note: You must exit the Calibration screen in order for the system to accept the calibration values.
- **Conductivity Flow Cell Constant Calibration**: Allows the user to calibrate the conductivity flow cell. The conductivity flow cell constant is printed on a tag attached to the flow cell cable. When a new conductivity flow cell is installed, use this value to enter the flow cell constant.
  Note: You must exit the Calibration screen in order for the system to accept the calibration values.
- **pH Probe Calibration**: This utility allows the user to calibrate the pH monitor. It is typically used at the start of each day's use of the system.
  Note: You must exit the Calibration screen in order for the system to accept the calibration values.
- **EGP Calibration**: This is informational only. It reminds the user to calibrate the pump through the pump software. Refer to the EGP Instruction Manual.

Table 5-7. Options Drop-down Menu

Options menu: Manual Screen



Options menu: Browser, Setup, Protocol, Post-Run Screen



* Time and Volume are displayed

The contents of the Options menu depends up the displayed screen as indicated above. These selections are used to change screen options.

- **Edit User Preferences**: Lets you specify the following:

  Run Options: Allows you to elect to have the the valves automatically return to position 1 at the end of a run.

  Protocol Editor Mode: This determines whether the default protocol is to be based on time or volume.

  Time Format: Allows you to select the time format (HH:MM:SS or min.tenth) used for the chromatogram X-axis.

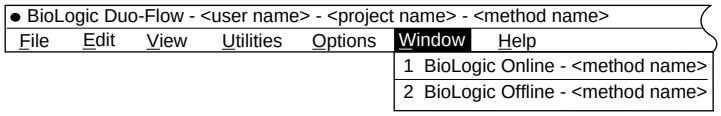
  BioFrac Tube Number Format: Allows you to select how the tube numbers are displayed on the Manual, Run and Post Run chromatograms when a BioFrac fraction collector is used. In Rack and Tube mode, tubes are identified by the rack that they are located in and by the order that the tubes were filled. In Rack and Grid mode, the tubes are identified by the rack they are located in and by their rack grid location. This feature is particularly useful for locating samples in microplates.

- **Chromatogram settings**: Allows you to select which instrument traces will be visible on the chromatogram. Up to 8 instrument traces are selectable from among the following: standard UV detector, conductivity monitor, pH, system backpressure, theoretical %B concentration, four QuadTec wavelengths, and detector traces acquired via the Signal Import Module (SIM). For the Manual screen, the X-axis (time) and the Y-axis (AU) ranges can be set. For the Run and Post Run screens, only the Y-axis range can be set.
- **Status Lines**: Allows the status information at the bottom of the screen to be toggled on or off.
- **Browser settings**: This enables you to select a heirarchy of how the Browser is to display users/projects/methods/ runs. For example, select Projects to display a top level folder called “Projects” which contains all projects in the database.
- **Use Time/Use Volume**: These are available only from the Protocol screen, when defining a new protocol.

The following selections are available from the Manual screen:

- **Manual Setup**: This enables you to set the pump purge flow rate. Additionally, you can set an EGP split time period, select a QuadTec time constant, and set the SIM parameters. For the F10 pumps, you can specify a maximum purge rate of 10 ml/min; for the F40 pumps, you can specify a maximum purge rate of 40 ml/min.
- **Buffer Blender Setup**: This is available only when the Maximizer is used with the system. This allows you to switch between buffer blending and non-buffer blending modes. In buffer blending mode, a window listing buffer systems available for use is displayed. From this window a user can enter one or two point pH corrections if needed.

Table 5-8. Window Drop-down Menu



The BioLogic DuoFlow software allows you to continue working while a run is in progress (Offline). A complete discussion of this function is provided in section 7.4.2, Working Offline.

- **BioLogic Online**: Allows you to observe and control the run in progress.
- **BioLogic Offline**: Allows you to write a protocol, analyze data, and export data while a separate run is in progress.

6.0 INTRODUCTION TO THE BROWSER SCREEN

The Browser is the organizational tool for Users, Projects, and chromatography data. It is a database that is displayed as a tree hierarchy which can be sorted by Users, Projects, Methods, and Runs. Figure 6-1 shows the layout of the Browser screen. A User must be selected or a new user added to the list and then selected in order to gain access to any program function except Manual. **All menus and toolbar functions will be grayed out and inaccessible until you select, or enter a User name in the Browser.**

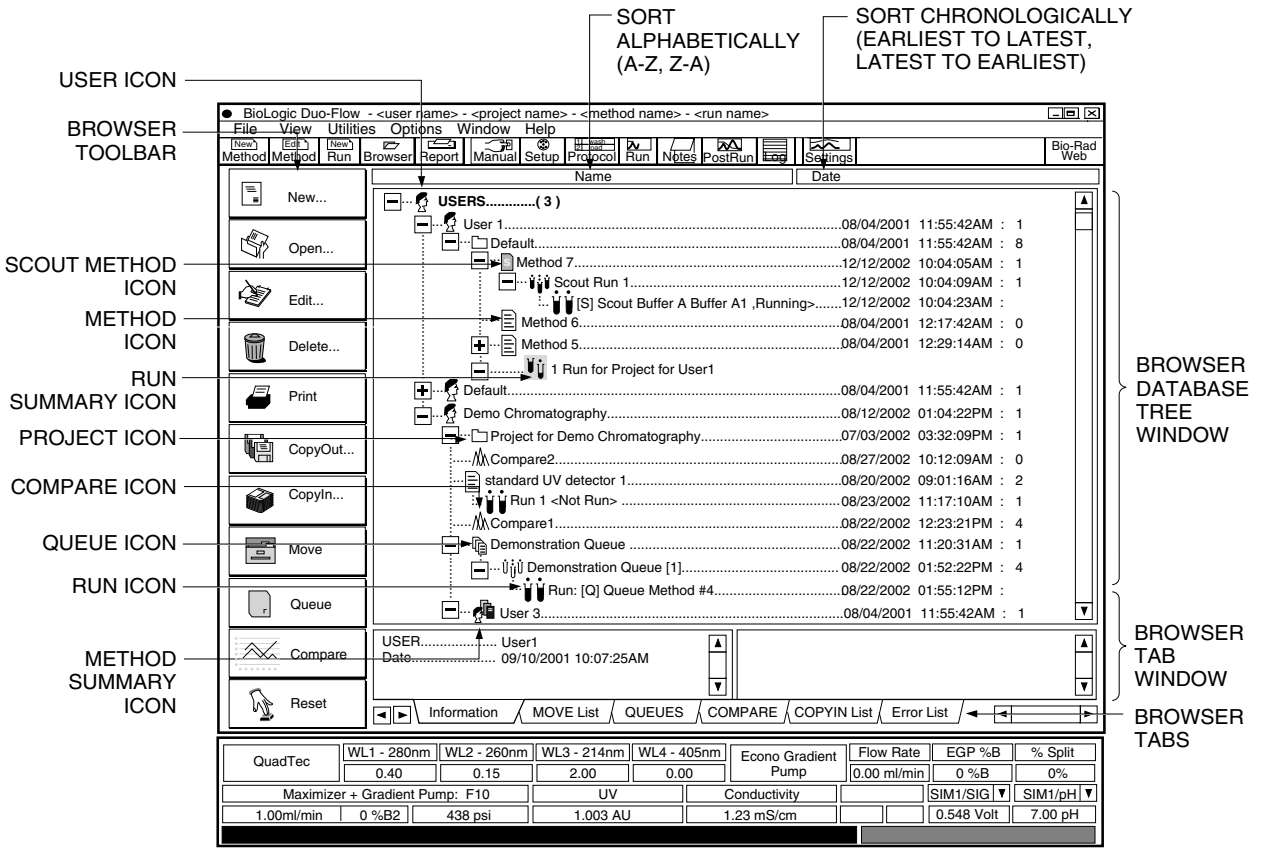


Figure 6-1. The Browser Screen

6.1 OVERVIEW

The Browser screen displays the following information and controls:

- **Collapsing/expanding the tree hierarchy:**

  [–] Click on this icon in the database tree to collapse the listing for a User, Project, Method, or Run folder.

  [+] Click on this icon in the database tree to expand the listing for a User, Project, Method, Queue, Scout or Run folder.

  Reset Updates and refreshes the Browser screen by collapsing all folders to the top Users icon.

- **Icon colors in the database tree indicate the following:**

  Green: Currently open in the active window. The active window may be the online window or the off-line window. For more detailed information about online and offline, refer to the discussion in Section 7.4.2, Working Offline.

  Red: Currently open in the window that is not active. This condition is displayed during off-line use: it applies to the method and run data for the window that is not active (online or offline). Items with a red check mark indicate that item is in the Move List or is marked for deletion.

  Blue: Currently not open in the Online or Offline window.

- **Bolded text in the database tree applies to the following:**

  Top level folders: Typically this applies to the USERS top level folder, which lists all Users in the system. By selecting Browser Settings from the Options menu, you can use "Starting Browser Selections" to display projects, methods, and runs on the top level of the tree hierarchy where they will be bolded.

  Summary information: The total number of methods are listed for each user, and the total number of runs are provided for each user project folder. You cannot delete summary information.

Browser screen controls and information

- **Browser toolbar**: Located down the left side of the screen, controls the different Browser functions. They are discussed in greater detail on the following page.
- **Name and Date bars**: Toggle buttons located across the top of the database tree that allow you to sort the tree alphabetically or chronologically. For example, you can click on the Name button to sort from a to z, and then click again to sort from z to a. If you click on the Date button to sort from the latest listing, then clicking again sorts from the earliest listing.
- **Browser Tabs and Browser Tab window**: The Browser tabs at the bottom of the Browser screen controls the information to be displayed in the Browser Tab window. The following tabs are available:

  Information tab: Displays two panels in the Browser Tab window. The left panel shows details about the specific item selected; the right panel displays a chromatogram when a run is selected.

  MOVE List tab: Displays the list of all items selected using the Move button to be moved from one place to another in the database list. Items selected to be moved are indicated by a red checkmark in the database tree. Projects can be moved from one user to another; methods can be moved from one project to another. When a project is moved, it takes all methods and runs associated with it; when a method is moved, it takes all runs associated with it. The procedure for moving an item is on page 6-5.

  Queue tab: Displays the methods in the Browser that have been placed in a Queue. The sequence of methods in a queue can be changed by dragging and dropping. Refer to Section 6.3 for running a queued method.

  Compare tab: Displays the runs selected or highlighted in the Browser for comparison. Refer to Section 6.4, for running Trace Compare.

  COPYIN List tab: Displays the list of all items selected to be copied in (restored) to the database from an archived data file or disk. The procedure for copying in items is discussed on page 6-4.

  Error List tab: Displays setup discrepancies that exist between methods in a Queue. These discrepancies must be corrected to run the queue.

Browser toolbar buttons

New... To create a new User, Project, Method, Run, Queue or Compare depending on which icon within the Browser is highlighted.

Open... Allows you to open a selected Method or Run from the database tree. Opening a Run allows you to view, analyze, and print the run data.

Edit... When a User or Project is selected, Edit allows you to change that User name or Project name and/or description.

When a Method or Run is selected, Edit copies the currently open method, renames it and places it in the Protocol Editor for editing. The new method is assigned a default name (<method name>.<version number>) that can be changed by the user.

Summary items are not editable.

Delete Eliminates Users, Projects, Methods and Runs from the Browser. To delete a Method, you must first delete all of the Runs associated with the method.

You cannot delete methods or runs from method and run summaries.

Print To generate and print the report for a run.

CopyOut... Allows you to copy Methods and Runs to floppy disk or to another location on your computer's hard drive. The CopyOut function is used for backup and archiving purposes and to transfer data between DuoFlow systems.

Copied out files are stored in a file with the default name biologic.zib, which is a compressed file format to conserve disk space. The copy out function copies the User, Project, and Method names associated with each.

Initiating a CopyOut to floppy disk completely erases the contents of the floppy disk.

To copy out, highlight the runs you wish to copy out. Press the **CopyOut...** button on the Browser toolbar. A dialog box appears that allows you to select the destination of the .ZIB file (either the hard drive or a floppy disk). Choose the destination and press **OK**.

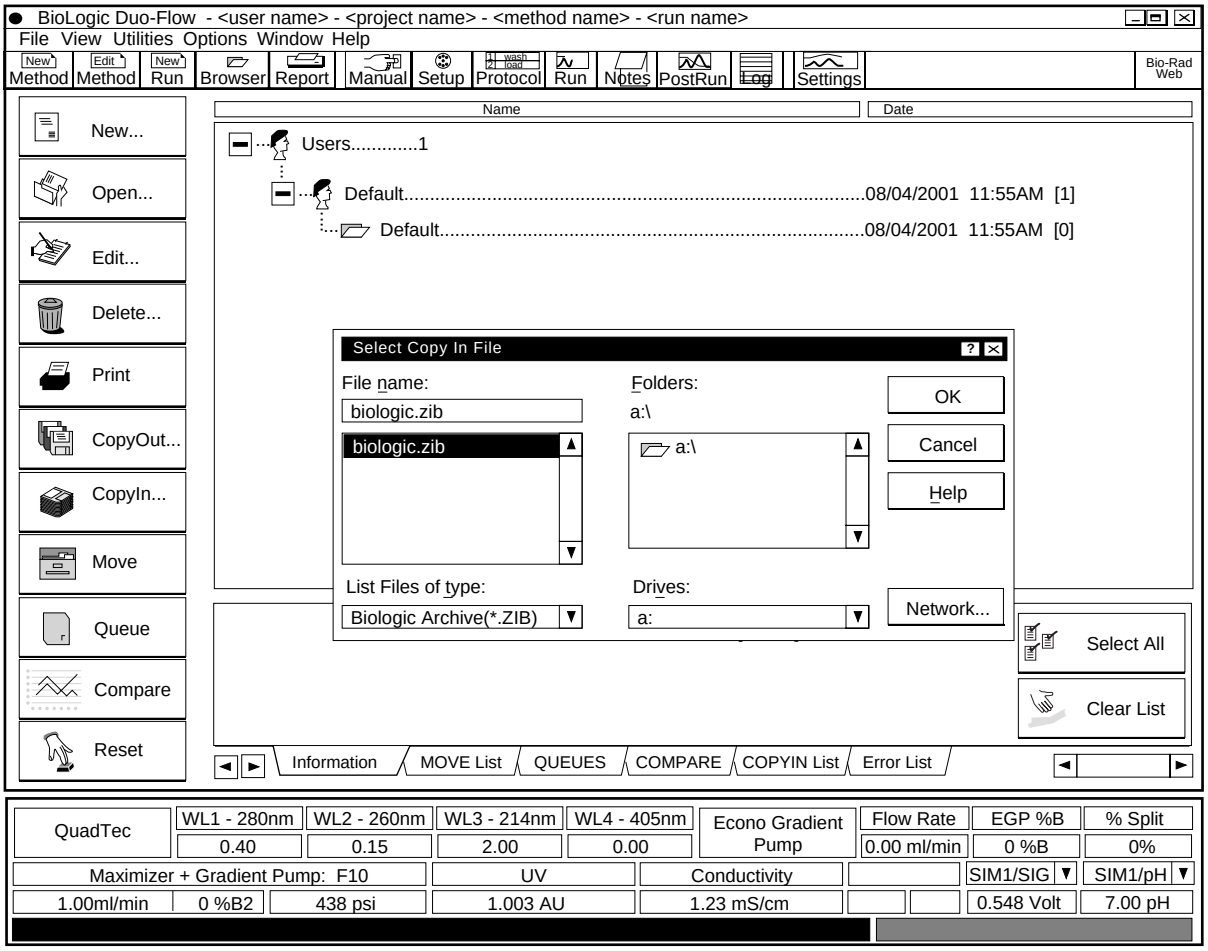


Figure 6-2. The CopyIn Window

CopyIn... Allows you to copy in Methods and Runs from a backed-up or archived .ZIB file. Refer to CopyOut page 6-3. To copy in:

a. Press the CopyIn... button and select the .ZIB file you wish to copy in (Figure 6-2 above.)

b. From the COPYIN List in the Browser Tab window, select the desired Methods and Runs. All Methods and Runs in the file are displayed in the COPYIN List (Figure 6-3.)

c. From the database tree window, click the left mouse button to select the Project to which you want the Methods and Runs to be copied.

d. Again, click on the CopyIn... button (or click on the right mouse button) and select Copy to <project name>. Specify where to copy the file to. Note that clicking the right mouse button displays all the available Browser toolbar options for the highlighted selection.

e. Once you finish the copy in procedure, use the Clear List button on the Browser tab window to delete the methods and runs in the COPYIN List.

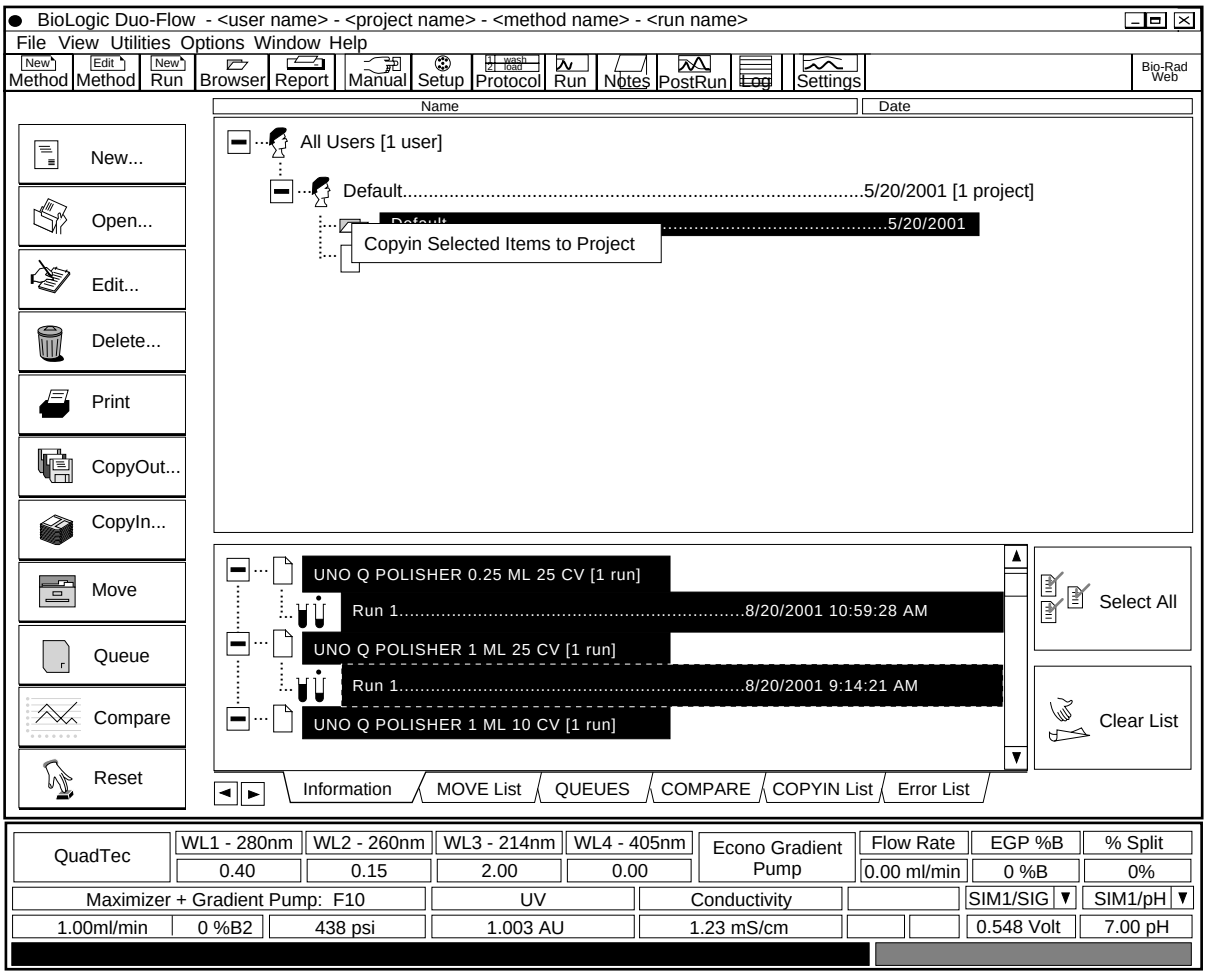


Figure 6-3. CopyIn with Information in Browser Tab Window

Move Allows you to transfer a selected Project or Method from one location to another. For example, a Project can be moved from one User to another, and a Method (and its runs) can be moved from one project to another. Individual runs cannot be moved. Not that the selected project, method and run have to be closed before they are moved. To move an item:

a. Highlight the item in the database tree you want to move. You may highlight a Project or a Method.

b. Click once on the Move button on the Browser toolbar. The items to be moved will appear in the MOVE List in the Browser tab window.

c. From the MOVE List, select the items you want to move.

d. From the database tree, select the new destination for the items you are moving.

e. Again click on the Move button. The items will automatically move to the destination. Hint: Alternatively, highlight the destination icon, right-mouse click and select the “Move Selected” message with a left-mouse click.

f. Once you finish the move procedure, use the Clear List button on the Browser toolbar to delete the remaining methods and runs in the MOVE List.

Queue Places methods in a queue. Highlight the method(s) you wish to place in a queue and select Queue. The methods will appear in the tab screen. Refer to Section 6.3 for detailed description of queuing.

Compare Places runs in a list for overlay comparison. Highlight the Run(s) you wish to compare and select Compare. The run will appear in the tab screen. See Section 6.4 for detailed description of trace compare.

Reset Updates and refreshes the Browser screen by collapsing all folders to the single user icon.

There are several options available for how information is displayed in the Browser screen. Select Browser Settings from the Options menu, discussed in Table 5-7, Options Drop-down Menu. The Set Browser Options window provides the following options:

- **Enable Projects**: A summary of all Projects, regardless of User, will be listed in the Browser.
- **Enable Methods**: A summary of all Methods, regardless of User or Projects, will be listed in the Browser.
- **Enable Runs**: A summary of all Runs will be listed in the Browser.
- **Enable User Methods**: A summary of all Methods for the specified User will be listed in the Browser.
- **Enable Project Runs**: A summary of all Project Runs will be listed in the Browser.

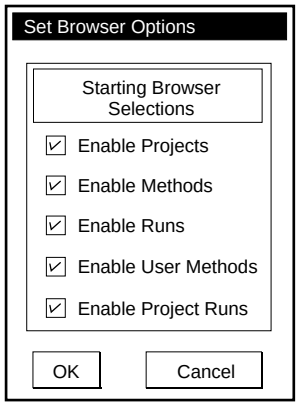


Figure 6-4. Set Browser Options Window

6.2 METHOD TEMPLATES

The BioLogic software includes Method Templates to simplify the method creation process. These templates can be used as is, or modified to fit experimental requirements. The templates that are available depend on the system configuration (BioLogic DuoFlow, BioLogic DuoFlow QuadTec, BioLogic DuoFlow Maximizer and BioLogic DuoFlow Pathfinder systems). Before using the Method Template feature, the system must be configured in the BioLogic Configuration utility and a QuadTec or UV detector should be defined in the default setup (see Section 7.2).

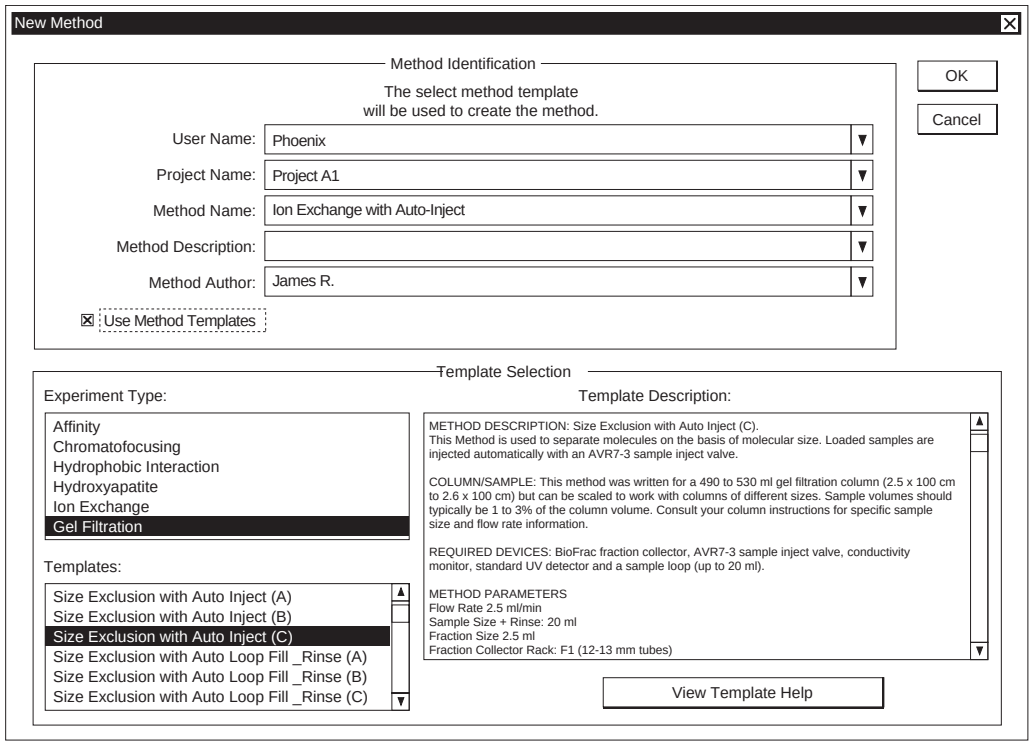


Figure 6-5. New Method Dialog Showing Method Templates.

To load a Method Template:

1. Select New/ New Method from the Browser toolbar or the New Method button on the system tool bar.
2. In the New Method dialog place a check in the "Use Method Templates" box (see Figure 6-5).
3. Select the Experiment Type
4. Select the Method Template. A brief description of each method template is given in the "Template Description" box, or a more detailed description can be viewed from the online help by pressing "View Template Help".
5. After entering the method name press OK to load the template.
6. If your system includes a Model 2128 fraction collector you will need to change the Method Template Setup. To this, go to the Setup Screen and delete the BioFrac fraction collector and replace it with a Model 2128. In the protocol screen open the fraction collection step and make sure the correct number of tubes is defined.

Procedures for creating a run in the Browser

1. Enter a New User.

Enter a new User to allow you to define your Methods and to group Projects, Methods, and Runs within the Browser.

a. Click once on the Users icon in the Browser.

b. Click once on the New... button and select New User. You will be prompted for a new User Name. Enter the name you would like to use.

c. Press OK to accept the new name.

If you define a new User, you must also assign a Project to the User before writing a new Method.

Hint: Alternatively, highlight a User icon, right-mouse click, and select New User with a left-mouse click.

2. Define a New Project.

The Browser is further segmented into Projects within a User's domain. To define a new project:

a. With the new User Name highlighted, click once on the New... button.

b. Select New Project. Enter the project name and description.

c. Press OK to accept the new name.

Hint: Alternatively, highlight the User icon, right-mouse click, and select New Project with a left-mouse click.

3. Write a New Method.

To begin writing a new method from the Browser:

a. Select the Project folder or a Method in the Project folder where you want the new method to be located.

b. Click once on the New... button.

c. Select New Method. Enter the method name and press OK. This transfers you to the hardware Setup screen.

Hints: **1.** Alternatively, highlight the Project icon, right-mouse click, and select New Method with a left-mouse click.

2. You can also use the New Method button in the toolbar. By default the system assumes that the current user and project shown in the window title bar is the path for the new method.

4. Name a New Run in a previously defined Method.

To start a new run from the Browser:

a. Select a Method or a Run in the Method where you want the new Run to be located.

b. Click once on the New... button.

c. Select New Run. Enter the Run name and press OK. The Run screen appears. Pressing the Start button in the toolbar will begin your sample run.

Hints: **1.** Alternatively, highlight the Method icon, right-mouse click, and select New Run with a left-mouse click.

2. You can also use the New Run button in the toolbar. By default the system assumes that the current User, Project, and method shown in the window title bar is the path for the new Run.

6.3 CREATING AND RUNNING A QUEUE

Queue allows you to run multiple methods in sequence. **Methods placed in a queue must have identical hardware setups or the system will not permit them to run.**

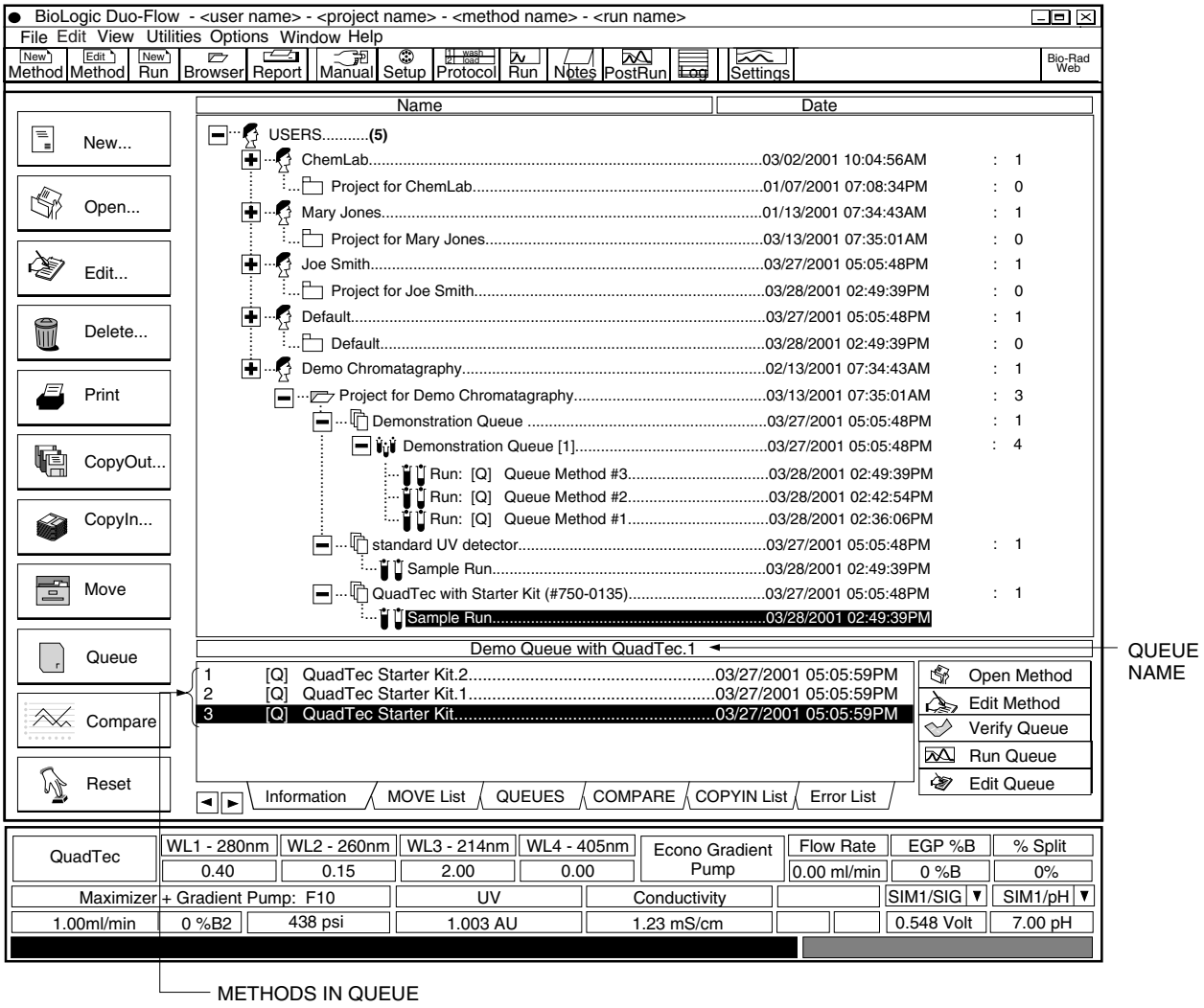


Figure 6-6. Queues Displayed in the Browser Window

To create a queue,

1. In the Browser screen select a User or enter a new User name.
2. Select a Project or enter a new Project name.
3. Select the NEW icon from the left screen sidebar, and from the displayed menu select New Queue. Enter a name and description for your Queue and click OK. Your queue name will be listed under your User and Project name, and the Queue tab will appear at the bottom of the screen. (See Figure 6-6 above.)

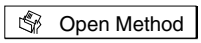
4. To place methods in a Queue, highlight the method in the Browser and click the Queue icon on the sidebar. Repeat until all desired methods are in the project Queue. This places each method into your Project Queue and the Queue Tab window at the bottom of the screen.

- Whenever you highlight the Queue icon under the Project Queue, the list of methods will appear in the Tab window.
- Multiple methods may be selected in the Browser by either shift/click or control/click.
- Methods will run in the sequence they are listed in the Tab window. The run order of the methods can be changed by dragging and dropping methods in the queue in the Tab window.

5. After all methods are listed in the project queue, click the Verify Queue button in the right sidebar of the Tab window to confirm that all methods meet the necessary criteria to be run.

- If the methods meet the necessary run criteria to run correctly, "Methods were successfully verified" will appear.
- If methods do not meet the necessary run criteria, a list of errors will appear. Errors will appear if methods are not compatible to run in sequence or the hardware Setup screens are not identical.
- To run your queue, click the Run Queue icon in the right sidebar of the Tab window.

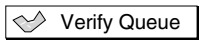
Description of the Queue Icons



Open Method: Opens a highlighted method for editing or viewing in the Protocol screen.

Delete Method: Deletes a highlighted method from the queue. This is displayed only prior to the queue running.

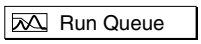
Edit Method: Copies the highlighted method, allows you to Rename it and then opens it for editing in the Protocol screen. This is displayed only when the Queue has completed its run. Queued methods cannot be edited after the run has started.



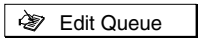
Verify Queue: Checks methods in a queue to assure there is not a conflict in the method Protocol and Setups to prevent running.

If there is a conflict, a list of errors will appear.

If methods are compatible, the message "Methods were successfully verified," appears.



Run Queue: Starts a run queue.



Edit Queue: Used to change the name and description of a Queue that has not been run or to make a copy of a Queue that has been run.

6.4 CREATING AND VIEWING A COMPARE

Trace Compare allows you to view and compare an unlimited number of chromatogram runs simultaneously in either tile or overlay mode.

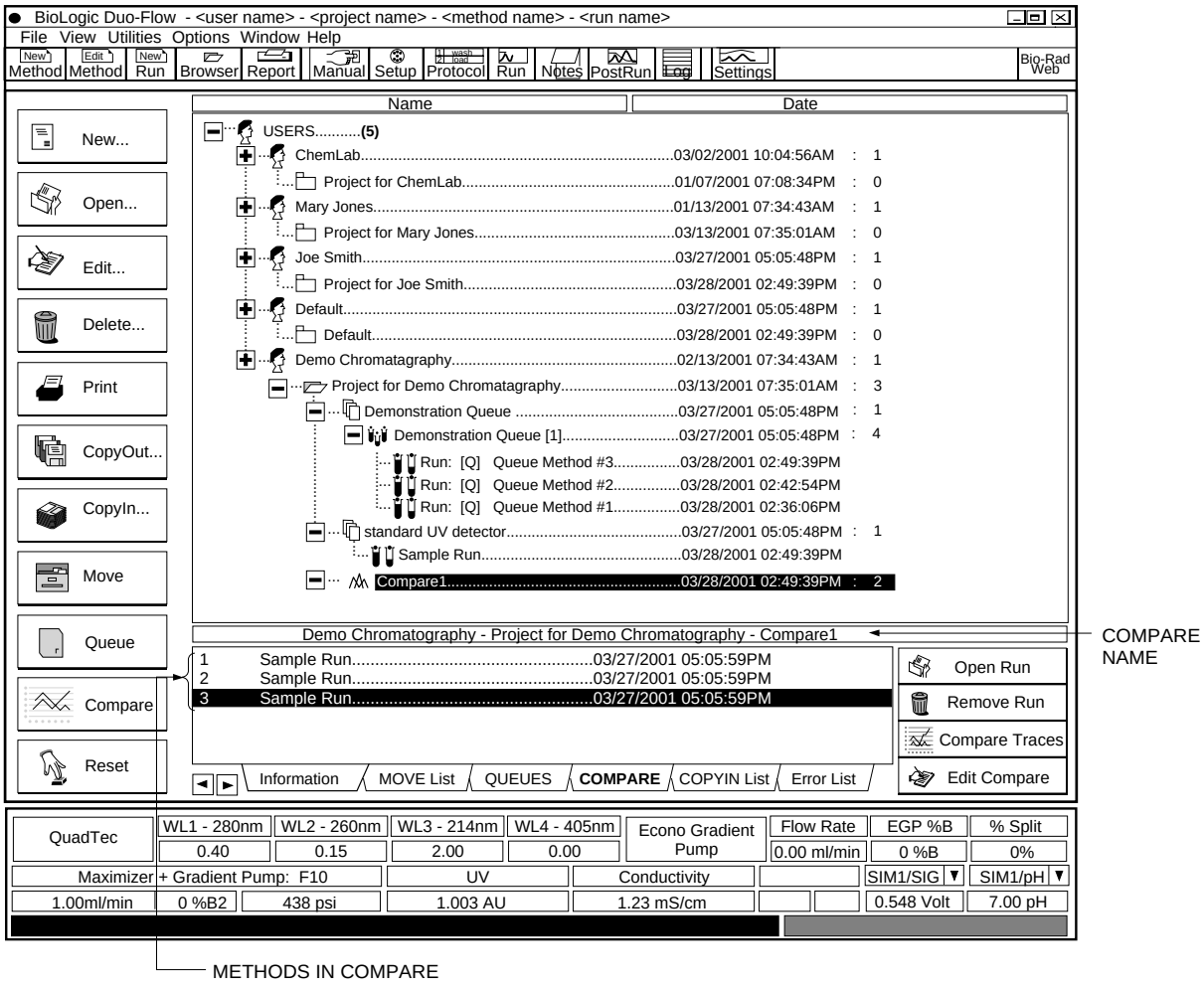


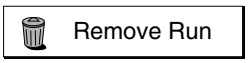
Figure 6-7. Compare Displayed in the Browser Window

To create a compare,

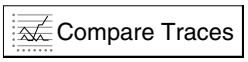
1. In the Browser screen select the user name and open the method that contains the runs you wish to compare.
2. Select the **NEW** icon from the left screen sidebar, and from the displayed menu select **New Compare**.
3. To place runs into the compare dialog, highlight the runs and click the **Compare** icon on the sidebar. Repeat until all desired runs are in the lower screen Project Compare window. This places each run into your Project Compare and the Compare Tab window at the bottom of the screen.
   - When you highlight the **Compare** icon under the Project Compare, the list of runs will appear in the Tab window.
   - Multiple methods may be selected in the Browser by either shift/click or control/click.

Description of the Compare Icons

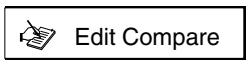
Open Run: Opens a highlighted run in the PostRun screen.



Remove Run: Deletes a highlighted method from the compare.



Compare Traces: Opens the Trace Compare window.



Edit Compare: Permits editing of the compare name, description and author.

6.5 TRACE COMPARE

Trace Compare is a tool used to simultaneously visualize and compare chromatography data. In Trace Compare run data can be compared side-by-side in Tiled mode or overlaid on top of each other in Overlay mode. The Trace Compare screen consists of five functional regions as described in the following Sections (see Figures 6-8 and 6-9).

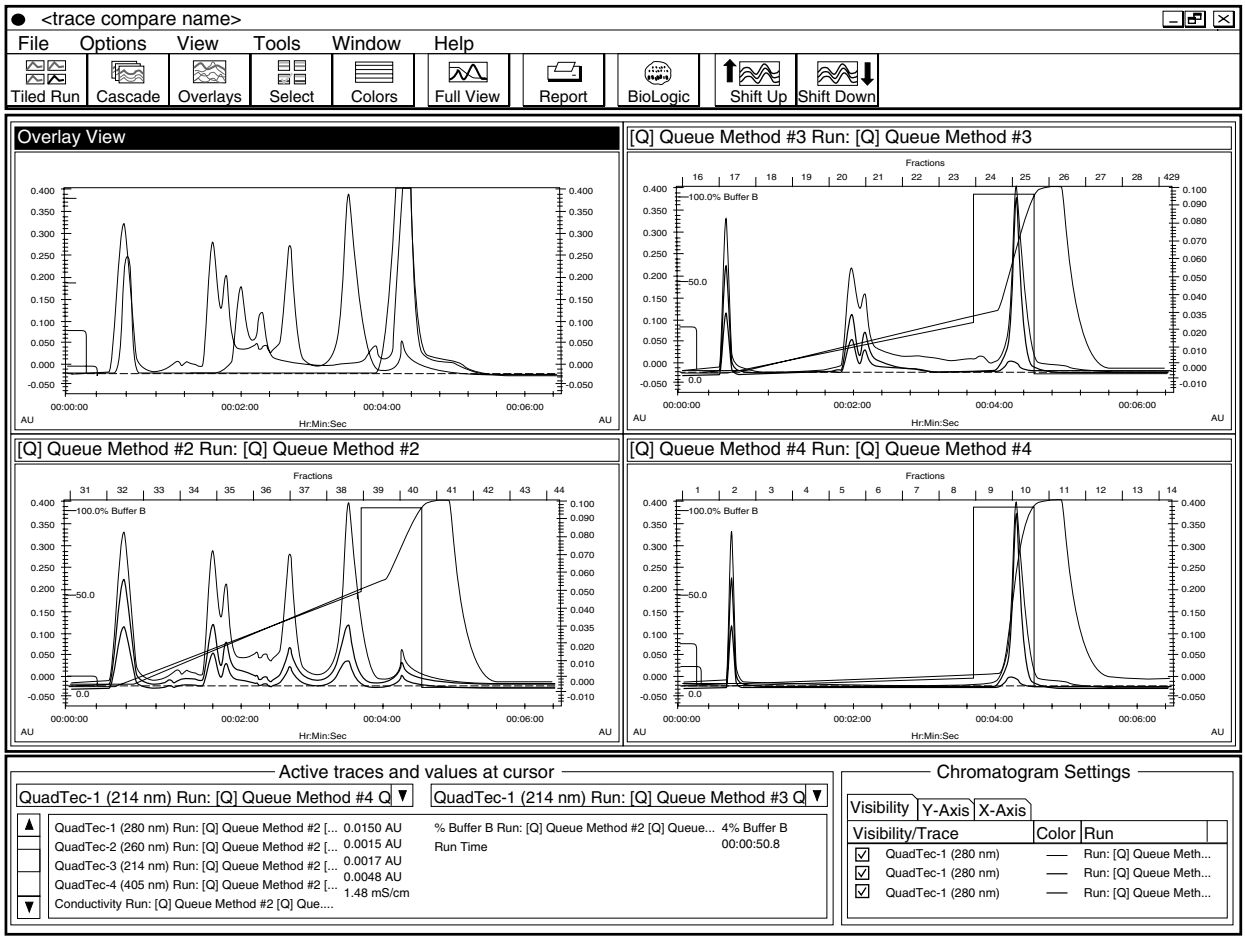


Figure 6-8. Trace Compare Window: Tiled View.

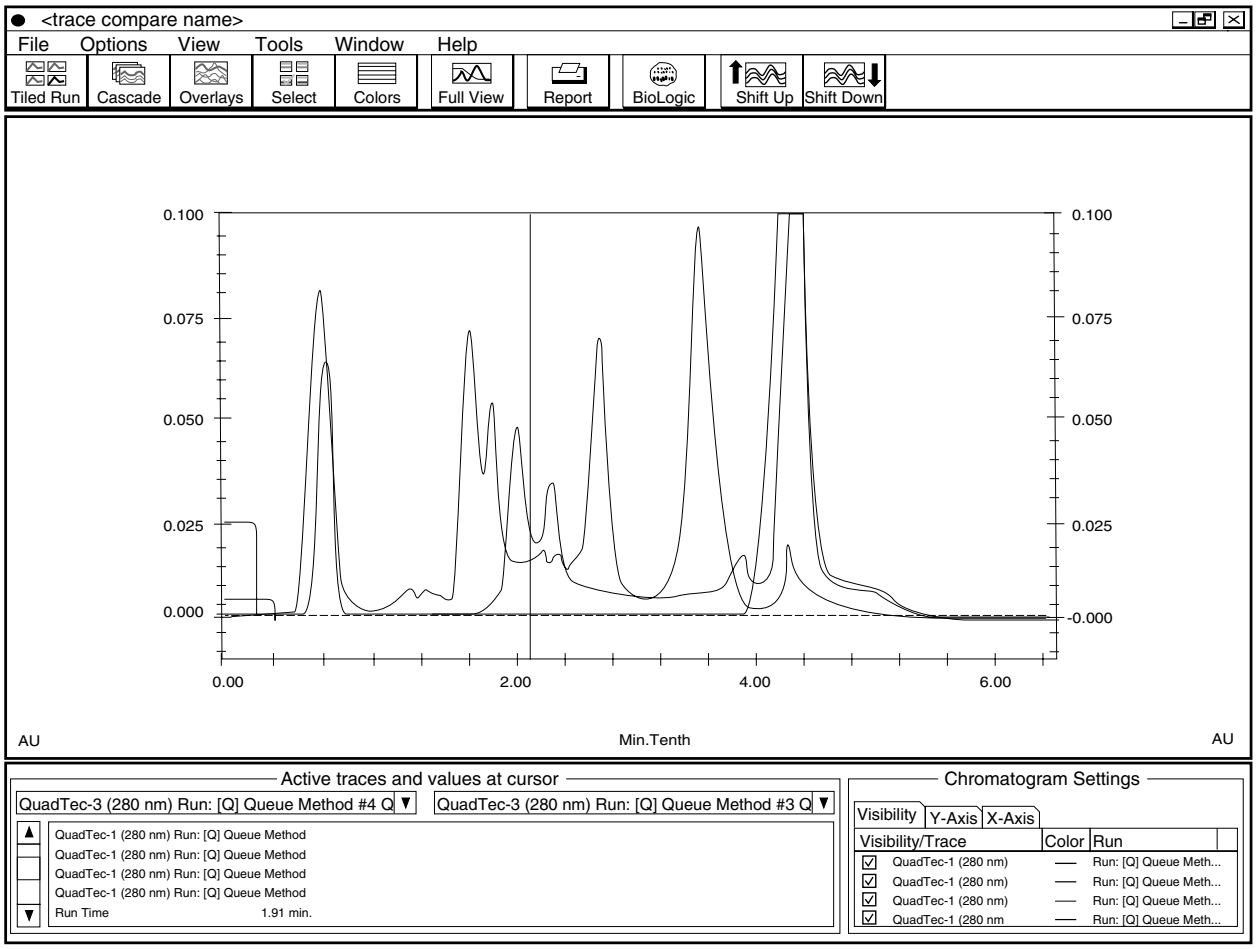


Figure 6-9. Trace Compare Window: Overlay View.

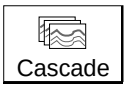
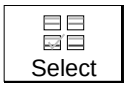
6.5.1 Chromatogram Display Screen

The Chromatogram Display Screen consists of one or more windows containing a chromatogram. Only one window is active at a time. The window appearance is controlled by the mouse as well as the Toolbar Buttons, Drop-down menus, "Active Traces and Values at Cursor" control and "Chromatogram Settings" control as described below. Windows may be resized by doing a click and drag on the side of the window with the mouse. Double clicking on the window title bar makes the window full screen. The window chromatograms can be zoomed by doing a click and drag with the mouse.

6.5.2 Toolbar Buttons

The function of each toolbar button is provided in Table 6-1.

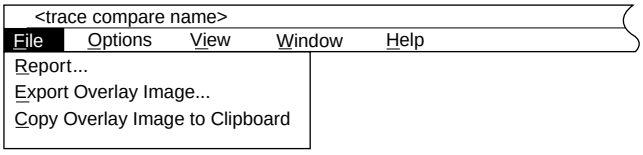
Table 6-1.
Toolbar Buttons

Button	Description
	**Tiled Run**: Displays Trace Compare chromatograms in in tile view.
	**Cascade**: Displays Trace Compare chromatograms in in cascade view.
	**Overlays**: Displays Trace Compare chromatograms in in overlay view.
	**Select**: Allows you to select which traces will appear in overlay view.
	**Color**: Toggles between two trace coloring schemes: Color by device type or color each trace uniquely.
	**Full View**: Expands the currently active chromatogram to full scale.
	**Report**: Allows you to preview and print a report of the currently open compare, including an Overlay Report, Trace Compare Report: Chromatograms, Trace Compare Report:Legends and Trace Compare Report: Summary.
	**BioLogic**: Closes Trace Compare and returns to the browser.
	**Shift Up**: Shifts chromatogram traces relative to each other in an upward direction.
	**Shift Down**: Shifts chromatogram traces relative to each other in a downward direction.

*Make sure that vertical placement of text is consistent throughout the Table.

6.5.3 Drop-down Menus

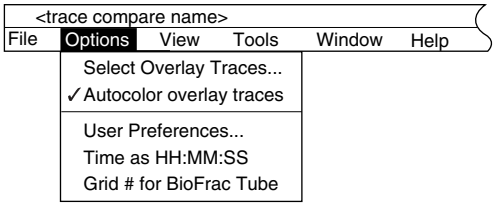
Table 6-2.
File Drop-down Menu



The File menu consists of the following:

- **Report**: Allows you to preview and print a report of the currently open compare, including an Overlay Report, Trace Compare Report: Chromatograms, Trace Compare Report:Legends and Trace Compare Report:Summary.
- **Export Overlay Image**: Allows you to export the overlay veiw to a windows metafile (.wmf) or enhanced metafile (.emf).
- **Copy Overlay Image to Clipboard**: Allows you to copy the overlay view to the clipboard for pasting into other applications.

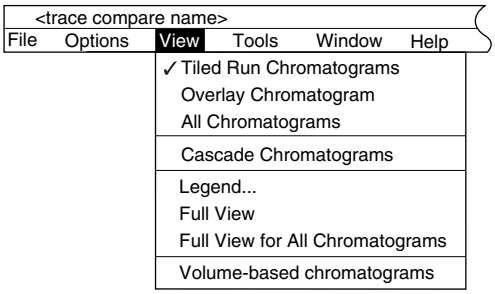
Table 6-3.
Options Drop-down Menu



The Options menu consists of the following:

- **Select Overlay Traces...**: Allows you to select which traces will appear in overlay view.
- **AutoColor Overlay Traces...**: Toggles between coloring each trace a different color or coloring each trace by its trace type.
- **User Preferences...**: Allows you to set the default time and BioFrac tube numbering format.
- **Time as HH:MM:SS / Time as Minute.tenth:** Toggles time format between HH:MM:SS and Minute.tenth.
- **Grid # for BioFrac Tube / Tube # for BioFrac Tube:** Toggles tube number format for the BioFrac between Grid numbering and Tube number.

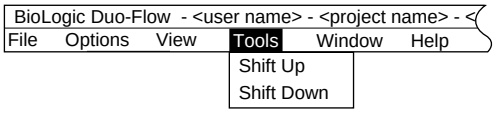
Table 6-4.
View Drop-down Menu



The View menu consists of the following:

- **Tiled Run Chromatograms:** Places Trace Compare in Tiled view and displays the run chromatograms side-by-side.
- **Overlay Chromatogram:** Places Trace Compare in overlay view.
- **All Chromatograms:** Places Trace Compare in Tiled view and displays all chromatograms including the overlay view side-by-side.
- **Cascade Chromatograms:** Displays chromatogram windows in cascase view.
- **Legend**: Displays the legend for the the current chromatogram.
- **Full View**: Expands the currently active chromatogram.
- **Full View for All Chromatograms**: Expands all zoomed chromatograms including the Overlay chromatogram.
- **Volume-based Chromatograms / Time-based Chromatograms:**

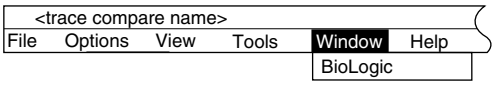
Table 6-5.
Tools Drop-down Menu



The Window menu consists of the following:

- **Shift Up:** Shifts chromatogram traces relative to each other in an upward direction.
- **Shift Down:** Shifts chromatogram traces relative to each other in a downward direction.

Table 6-6.
Window Drop-down Menu



The Window menu consists of the following:

- **BioLogic:** Closes Trace Compare and returns to the Browser screen.

6.5.4 Active Traces and Values at Cursor

The Active traces and values in the “at cursor” window located in the upper left of the post run screen, contain two drop-down menus that control which trace axis is displayed on the left and right sides of the active chromatogram. The scroll bars on the left and right sides of the information box control the scaling of the trace associated with the left or right drop-down menu. When the vertical crosshatch bar is dragged across a chromatogram, the values relating to the baseline or peak underneath the crosshatch is reflected in the upper left corner of the screen. The value for each trace at the cursor position is shown in the dialog.

6.5.5 Chromatogram Settings Tab

The Chromatogram Settings window contains the following tabs to manipulate the display of the chromatogram:

Visibility	In Tile mode each trace device type is displayed along with a check box, legend, and a run name. Checking or unchecking the boxes causes the traces to be displayed or hidden. In Overlay view only the traces selected in "Options/Select Overlay Traces..." or by the **Select** button are shown in the chromatogram settings dialog. The currently active trace is highlighted in blue.
Y-Axis	Allows you to set the Baseline and Axis Max values for the currently active trace. The active trace is selected in the Visibility tab.
X-Axis	Allows you to set the Start Time and End Time for the currently active chromatogram.

7.0 MODES OF OPERATION

There are two primary modes of system operation: the user can operate the system manually (from the Manual screen) or through the use of a user-defined method. Each of these modes of operation is discussed below, as well as the screens involved in setting up, defining, and running a method.

- Operating the system using the Manual screen: This allows you to individually control the devices and instruments connected to the Workstation. Examples of its use include purging the pumps and equilibrating the columns. For further discussion, refer to Section 7.1.
- Operating the system using a user-defined method: This involves the following:

  New Method: Allows you to create a method from scratch or load a Method Template.

  Setup screen: Allows you to define the instruments and devices to be used for any method.

  Protocol screen: To define each of the steps to be run during a method. Most steps are programmed using a dialog box to enter the parameter values. Note that the number of the step being programmed is displayed in the upper left corner of the dialog box.

  Run screen: To run a method or view a run in progress.

  Start: Starts a selected run.

  Note: You may move through the four screens by activating buttons on the system toolbar as long as a run is not in progress. Those functions available during a run are discussed in section 7.4, Run Screen.

The relationship between the the two modes of operation, as well as the screens involved in defining and running a method, is illustrated in Figure 7-1 below.

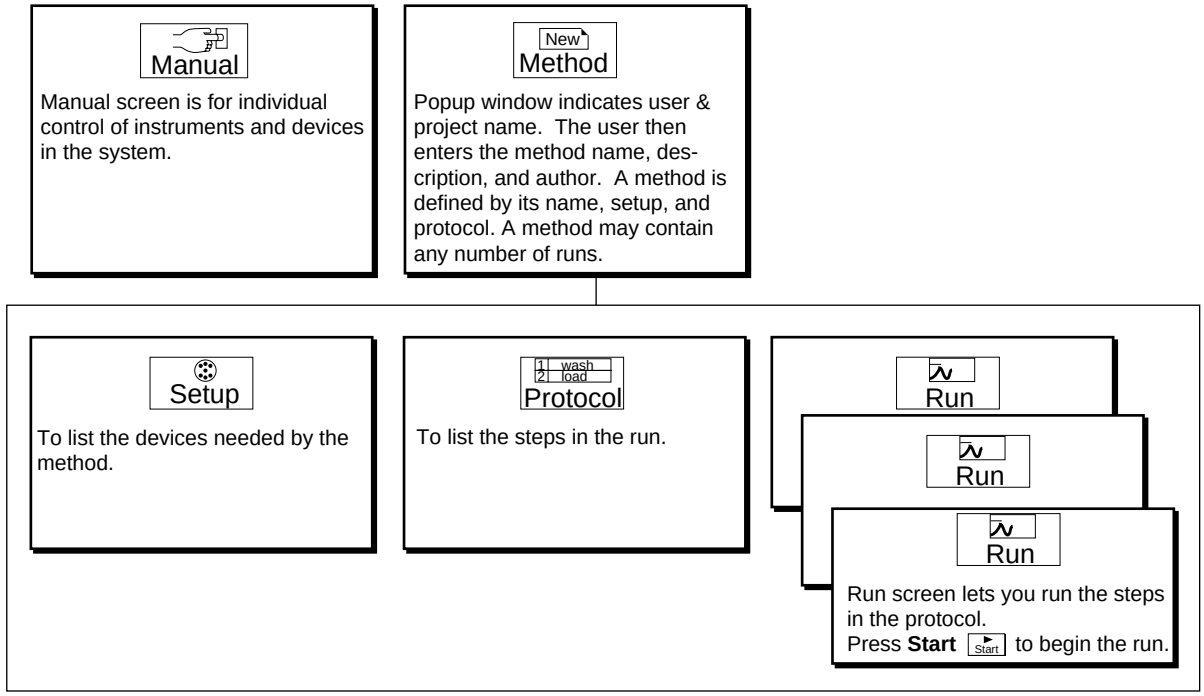
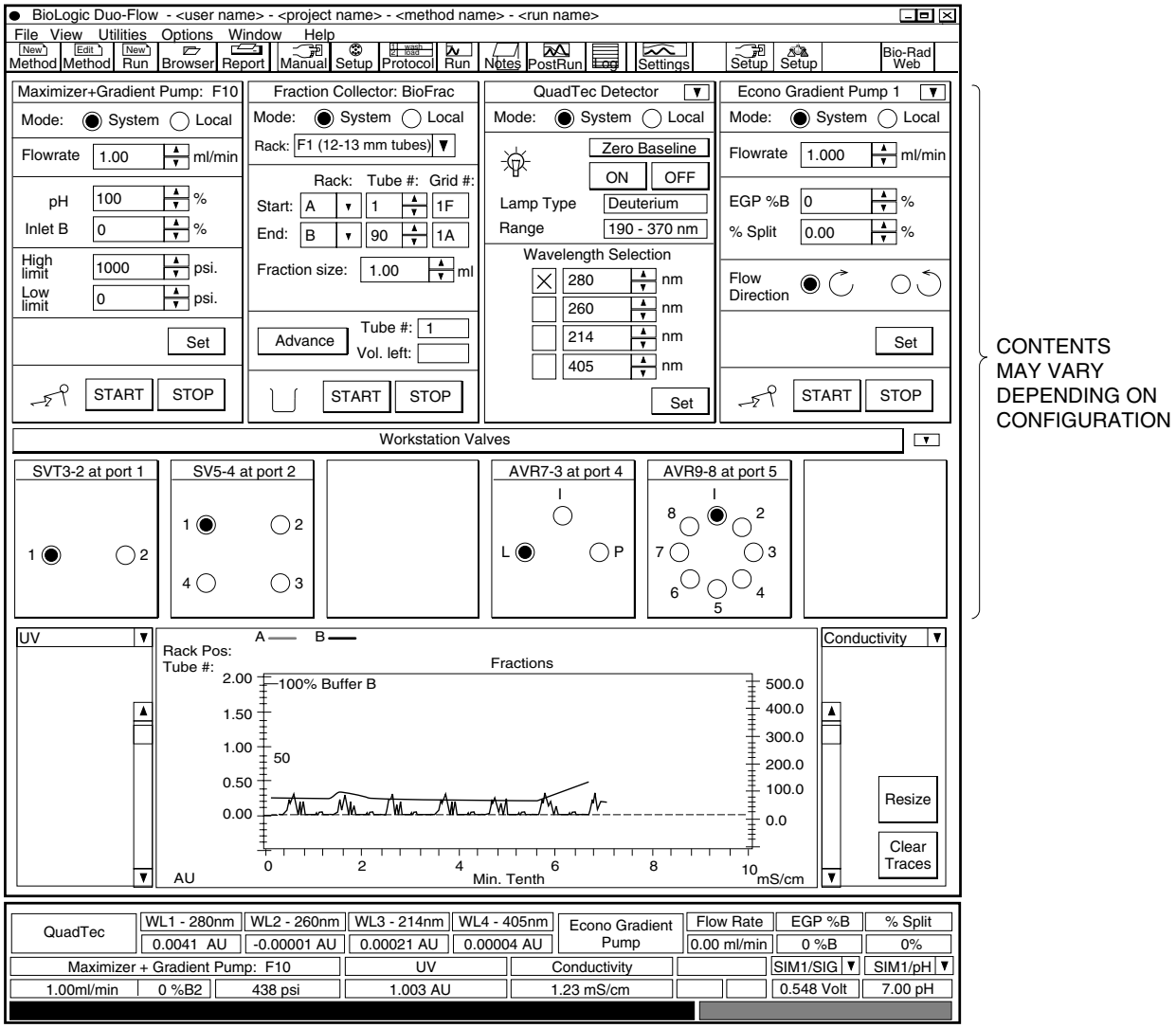


Figure 7-1. Relationships between Modes of Operation

7.1 MANUAL SCREEN

The Manual screen is used to control and monitor the operation of the DuoFlow Workstation and each of its peripheral devices. This interface is divided into three basic groups of dialogs and includes control panels, a real time chromatogram display and a status bar.



CONTENTS MAY VARY DEPENDING ON CONFIGURATION

Figure 7-2. Manual Screen, for the BioLogic DuoFlow system connected to a Maximizer, BioFrac Fraction collector, QuadTec Detector, Econo Gradient Pump, and Four Valves

From the Manual screen you can control the following:

Gradient Pump: This panel is used to control buffer composition, flow rate and pressure limits (see Table 2-3) as well as to start and stop the pumps. The Start/Stop buttons are used to start and stop the pumps. The Set button is used to make changes to the composition, flow rate or pressure limits while the pumps are running.

Maximizer + Gradient Pump: This panel is displayed in place of the Gradient Pump panel when a Maximizer is connected to the system and is used to control buffer composition, flow rate and pressure limits (see Table 2-3) as well as to start and stop the pumps. When Buffer Blending is turned on, composition is determined by pH and % Inlet B. When Buffer Blending is turned off, composition is determined by percent Inlet A1 or A2 and percent Inlet B1 or B2. The Start/Stop buttons are used to start and stop the pumps. The Set button is used change the composition, flow rate and pressure limits while the pumps are running. The System and Local option buttons are used to toggle the Maximizer between System and Local mode.

Fraction Collector: This panel is used to control a fraction collector. Its appearance depends on the type of fraction collector connected to the system (BioFrac or Model 2128). From this panel the Start Tube, End Tube, Fraction size and rack type are set and the fraction collector can be started or stopped. Also displayed on this panel are the current "Tube number" and the tube "Volume left" to fill. The System / Local option buttons are used to toggle a BioFrac or Model 2128 fraction collector between System and Local mode. Only fraction collection by volume is supported in manual mode. When a BioFrac is connected to the system the rack grid numbers for the start and end tubes are also displayed.

UV Detector / Chart Recorder / Signal Import Module: This panel is used to control the standard UV monitor and chart recorder as well as to monitor the status of the two signal import modules (SIM 1 and SIM 2), if they are connected to the they system (see Chapter 2.9.6 for more information). The UV detector panel is used to turn the Standard UV detector lamp on and off and to zero the baseline. The Chart Recorder panel is used to start/stop the chart recorder and to set the conductivity and UV range. Event marks can be added to the chart recorder output by pressing the "Event mark" button. The down arrow in the upper right corner of the panel is used to toggle between the QuadTec and UV detector control panels.

QuadTec Detector: This Panel is used to control the QuadTec detector. From this panel, the lamp type and wavelength range is viewed, the QuadTec detector can be turned on or off, up to four wavelengths can be selected and the UV-Vis baseline can be zeroed. For best performance, the QuadTec wavelengths should be entered in numerical order, starting in the upper most field. The Set button is used to accept any changes made in the Wavelength Selection boxes. The down arrow in the upper right corner of the panel is used to toggle between the QuadTec and UV detector control panels. The System / Local option buttons are used to toggle the QuadTec between System and Local mode.

Econo Gradient Pump: This panel allows you to control an Econo Gradient Pump (EGP) if it is connected to the system bit bus. From this panel, you can control the flow rate, % split (see Chapter 2.8.3) and flow direction as well as start and stop the pump. For more detail see the EGP instruction manual.

Workstation Valves / Maximizer Valves: These two panels are used to control any of the solenoid and automated valves connected to the workstation and Maximizer. The down arrow in the upper right corner of the panel is used to toggle between the workstation and Maximizer valves.

Chromatogram: This panel is used to view up to 8 instrument traces during a manual run. Traces may include: UV detector, conductivity, pH, pressure, theoretical %B, four QuadTec wavelengths, and signals from two external detectors (see Chapter 2.9.6 and Table 2-7 for more information about the SIM-HR and the Maximizer SIM, respectively). The chromatogram display is controlled through a combination of the Chromatogram Settings dialog (Settings button or Options pull-down menu), the Resize and Clear Traces buttons and the scroll bars. Each scroll bar is assigned to an individual trace by the drop-down menu above it.

Status Bar: This panel is used to display numerical data from the BioLogic DuoFlow workstation and its attached devices. Parameters include: gradient pump flow rate, %B and pressure; UV detector absorbance, QuadTec UV-Vis wavelength and absorbance; conductivity; SIM data (from a pH probe or an external detector) as well as the Econo Gradient Pump flow rate, %B and % Split.

7.2 SETUP SCREEN

The Setup screen (see Figure 7-3) is used to select instruments and devices for use in a user-defined method, name (alias) the Workstation and Maximizer inlets and create Buffer Blending buffer systems (Buffer Editor). Instruments and devices may include: fraction collector (BioFrac, Model 2128, Model 2110 or generic (non-Bio-Rad)); detector (Standard UV, QuadTec UV-Vis, pH, Conductivity, SIM); Valves (SVT3-2, SV5-4, AVR7-3, AVR9-8), auxiliary pump (Econo Gradient, Econo EP-1 or a generic (non-Bio-Rad) pump) and a Buffer Blender. When a new method is created, the devices defined in the default setup are automatically loaded into the "Devices in setup" list. For this reason, devices that are used routinely should be saved as part of the default setup with the File/Save Setup function. Furthermore, the Method Template feature determines the type of detector (Standard UV or QuadTec) that should be included in each template from the default setup.

Devices are added to the setup using the "Available Devices" buttons shown in Figure 7-3. When a device is selected, a dialog is displayed that allows device specific parameters to be entered. When the maximum allowed number of a device has been added to the "Devices in setup" list, the corresponding "Available Devices" button becomes grayed out. Devices may be removed from the "Devices in setup" list by pressing the toolbar Delete button. Double-clicking a device in the "Devices in setup" list opens a dialog used to edit the device settings.

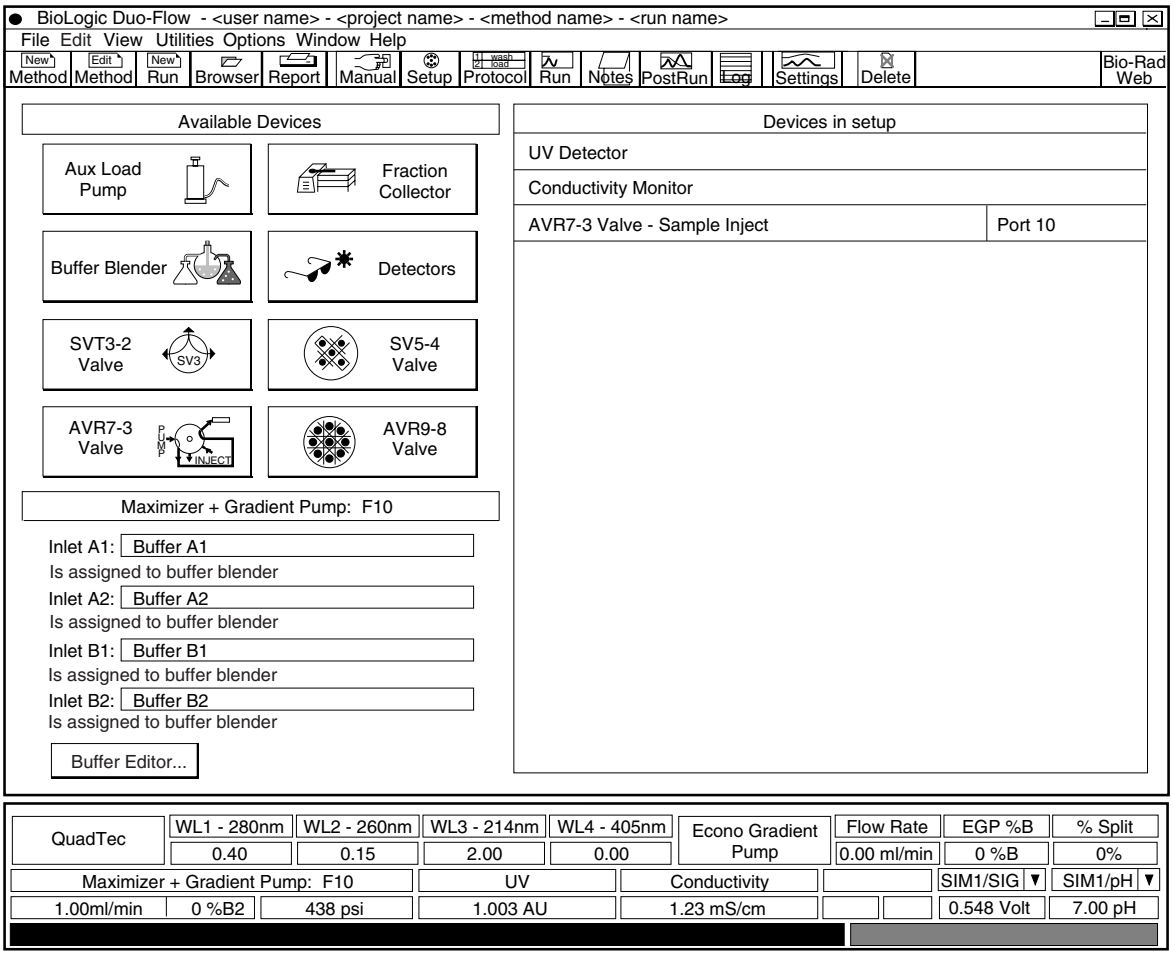


Figure 7-3. Setup Screen

7.2.1 Device Selection

The devices that can be defined in the device setup are described below. The actual devices available on your DuoFlow system may vary. All DuoFlow systems are delivered with a detector, conductivity monitor and an AVR7-3 valve. All other devices are optional (See Appendix D for information about ordering additional devices).

- **Auxiliary Pump**: Used to define the type of auxiliary pump connected to the DuoFlow system: Econo Gradient Pump (EGP), Model EP-1 Econo or generic (non-Bio-Rad) pump. These pumps may be defined as a sample load pump or as a user-defined pump (EGP only). See Chapters 2 and 8 for ways to use an auxiliary load pump. Generic pumps should be defined as a Model EP-1 pump.
- **Fraction Collector**: Used to define the type of fraction collector connected to your system: BioFrac, Model 2128, Model 2110 or generic (non-Bio-Rad). The fraction collection options available depend on the type of fraction collector connected to the system. All of the fraction collectors can be synchronized with a detector signal by checking "Synchronize Fraction Collection with the Detector" and entering a delay volume. The delay volume is defined as the system fluid volume (including tubing and inline devices) between the significant detector and the fraction collector drop head. See the BioLogic software online Help for help determining the delay volume.

  The BioFrac and Model 2128 options are used to control these fraction collectors over the system bus and to set the rack type, collection pattern (BioFrac only) and whether or not the maximum number of racks may be exceeded. If you choose to exceed the maximum number of available racks, the run will pause when the rack(s) are full and prompt you to add new racks. The BioFrac or Model 2128 fraction collector must be selected in the BioLogic Configuration Utility (see Chapter 3.11.3) before starting the BioLogic software.

  The Model 2110 and generic options allow you to control these fraction collectors over the Aux port on the rear of the BioLogic Workstation or Maximizer. If a Maximizer is connected to your system then the fraction collector must be connected to it and not the Workstation. Note, a SVT3-2 valve must be connected to your system and defined as a diverter valve in the device setup if threshold or windows collection is desired.
- **Buffer Blender**: Used to place the DuoFlow system in Buffer Blending mode, select a pre-defined Bio-Rad or user-generated buffer system, set pH corrections and view solution preparation instructions, pH range and buffer temperature coefficients. The Buffer Editor feature is used to generate user-defined buffers for Buffer Blending (see Chapter 7.2.3).
- **Detectors**: Used to define the detectors types used by a method. Up to six detector traces may be defined including: UV Detector, QuadTec (up to four traces), Conductivity, SIM pH and SIM signal (up to two external detector traces such as refractive index or fluorescence detectors). The external detectors are connected through a SIM-HR and/or a Maximizer SIM (see Chapter 2.9.6 and Table 2-7, respectively).
- **Valves (SVT3-2, SV5-4, AVR7-3 and AVR9-8)**: Used to define valve functionality for 3 solenoid and 3 motorized valves (6 solenoid and 6 motorized valves if a Maximizer is installed with your system). The valve setup dialogs are used to define the valve name and function, specify which port the valve is connected to and to assign a name/function to each valve position. See Table 7-1 for valve function options.

7.2.2 Inlet and Valve Naming

One of the DuoFlow Software's most powerful features is the ability to define a valves function and to name inlet and valve positions. This greatly simplifies programming individual steps in the Protocol Editor. Each DuoFlow inlet (A, B, or A1, A2, B1, B2 on a system configured with a Maximizer) can be assigned to a specific valve (Inlet Valve) or be assigned to a specific buffer. The "Gradient Pump" and "Maximizer + Gradient Pump" section of the Setup screen is used to assign Inlets A and B (A1, A2, B1 and B2 on a system with a Maximizer) to a specific buffer. Alternatively, the SVT3-2, SV5-4 and AVR9-8 valves can be assigned as Inlet valves and each port of the inlet valves assigned to a specific buffer.

Table 7-1.
Valve Setup Information

Valve Type	Valve Name/ Function	Position Names	Notes
SVT3-2 Low pressure solenoid valve	Fraction Collector Diverter	1 Waste 2 Collect	Functions as a fraction collection diverter, determined by the actual fraction collection parameters chosen.
Note: Indicate the location of the valve cable connection.	Inlet A	Named by user	When used before the inlet to Pump A or B, the valve enables buffer selection. The buffer name specified for each position will appear in the Protocol screen's "Isocratic Flow", "Linear Gradient", and "Change Valve" dialog box. Refer to Chapter 8, Sample Loading, for examples.
	Inlet B	Named by user	
When the Maximizer is used, inlets A and B become A1, A2, B1, B2.	Aux Pump Inlet	Named by user	Used for auxiliary pump load selection to select one of two solutions. Refer to Chapter 8, Sample Loading, for examples.
	User Assigned Name	Named by user	When used for a purpose other than described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box.
SV5-4 Low pressure solenoid valve	Inlet A	Named by user	When used before the inlet to Pump A or B, the valve provides preparative sample loading or buffer selection. The buffer or sample name specified for each position will appear in the Protocol screen's "Isocratic Flow", "Linear Gradient", and "Change Valve" dialog box. Refer to Chapter 8, Sample Loading, for examples.
Note: Indicate the location of the valve cable connection.	Inlet B	Named by user	
	Aux Pump Inlet	Named by user	Used for auxiliary pump load selection to select one of four solutions. Refer to Chapter 8, Sample Loading, for examples.
When the Maximizer is used, inlets A and B become A1, A2, B1, B2.	User Assigned Name	Named by user	When used for a purpose other than that described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box.
AVR7-3 High pressure valve	Sample Inject	1 Load Sample 2 Inject Sample 3 Purge	For automatically loading a sample.
Note: Indicate the location of the valve cable connection	User Assigned Name	Named by user	When used for a purpose other than described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box. Refer to Section 4, Advanced System Applications, Chapters 8 through 10.

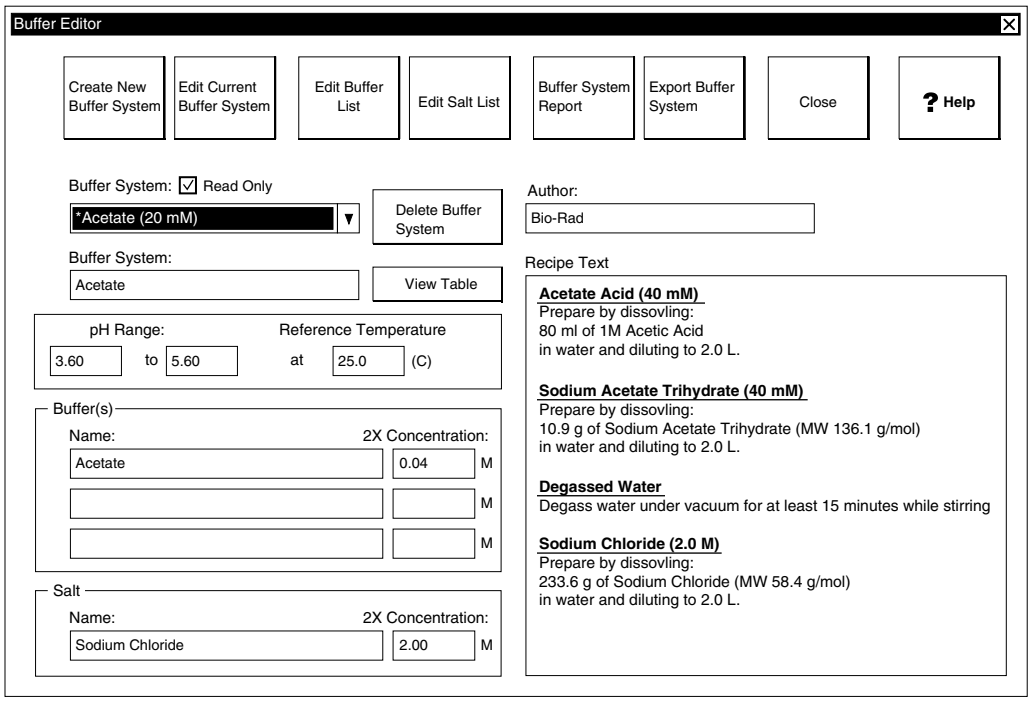
Table 7-1. (continued)
Valve Setup Information

Valve Type	Valve Name/ Function	Position Names	Notes
AVR9-8 High pressure valve	Aux Pump Inlet	Named by user	Used for auxiliary pump load selection to select from up to eight samples, buffers, or rinse solution.
Note: Indicate the location of the valve cable connection. When the Maximizer is used, inlets A and B become A1, A2, B1, B2.	Inlet A	Named by user	When used before the inlet to Pump A or B, the valve provides preparative sample loading or buffer selection. The buffer or sample name specified for each position will appear in the Protocol screen's "Isocratic Flow", "Linear Gradient", and "Change Valve" dialog box. Refer to Section 4, Advanced System Applications, Chapters 8 and 9.
	Inlet B	Named by user	
	Fraction Collector	1 Waste 2 Collect 3-8 Named by user	Useful for collecting large volume samples. Collects up to 8 samples. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box.
	User Assigned Name	Named by user	When used for purposes other than described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box. Refer to Chapters 8 and 9.
	Column Switching	Named by user	Allows you to install up to 8 columns and assign valve numbers and names to each column. Requires two AVR9-8 valves: one is an inlet valve and the other functions as an outlet valve.

7.2.3 Buffer Editor

The Buffer Editor is used to create new Buffer Blending buffer systems from user-supplied information. This information is used by the Maximizer to determine the amount of acid, base, water and salt required to produce a buffer solution at a desired pH and salt composition. Each buffer system can include up to three buffers and each buffer can have up to three pKa's. The Buffer Editor also generates a recipe describing how to make each Buffer Blending solution. The Buffer Editor requires information about the buffer (pKa(s), temperature coefficient(s), charge state, molecular weight and concentration) and the salt (charge state molecular weight and concentration). This information can also be supplemented with user determined 1 or 2 point pH correction for each buffer in a buffer system. pH corrections are entered from the Buffer Blending setup dialog.

Table 7-2
Buffer Editor



The **Buffer Editor** main screen is used to view information about currently defined buffer system and to create and edit buffer systems.

- **Create New Buffer System**: This button starts the Buffer System Wizard that is used to create new buffer systems. Buffers and salts that are defined in the current buffer and salt lists can be used in a buffer system.
- **Edit/View Buffer System**: This button starts the Buffer System Wizard and is used to edit, view or copy existing buffer systems. Buffers and salts that are in the current buffer and salts lists can be used in a buffer system.
- **Edit Buffer List**: This button starts a dialog used to create buffer salts that are used in buffer systems.
- **Edit Salt List**: This button starts a dialog used create salts that are used in buffer systems.
- **Buffer Editor Report**: This button allows you to print out buffer system information.
- **Export Buffer System**: This button allows you to export buffer system information to a text file.
- **Close**: Exits the Buffer Editor.
- **Help**: Opens the Buffer Editor online help.
- **Delete Buffer System**: Deletes the currently selected buffer system. Bio-Rad buffer systems cannot be deleted.
- **View Table**: Used to display the pH as a function of % Inlet A2 and %B. Used to determine the pH range for a buffer. The buffer pH range depends on the salt concentration and should be limited to the table pH valves shown between 10% and 90% A2.

7.3 PROTOCOL SCREEN

The protocol screen is used to create a new method or edit an existing method. Isocratic and linear gradient step duration may be programmed in units of volume or time. The default duration units (time or volume) can be set using the drop-down menu item: Options/Edit User Preferences. The menu items Use Time (min) and Use Volume (ml) may be used to toggle between the two modes.

- **Add Step buttons**: These buttons are used to add steps to the protocol. They are located in a scroll box on the left side of the protocol screen. To insert a step, highlight the step below where the new step is to be added and then press the appropriate button to define the new step (see Tables 7-3 through 7-9 for more information). The buttons that are active depend on which devices have been defined in the device setup.
- **Fraction Collector button**: This button is used to define the fraction collection scheme used by the protocol. It is located below the Add Step buttons on the protocol screen (see Table 7-10 for more details). This button is active if a fraction collector has been defined in the device setup.
- **Scout Button**: This button is used to convert the currently defined method into a scout method. Note that the method cannot be edited once a scout has been defined unless the scout is deleted (see Table 7-11).
- **Edit buttons**: These toolbar buttons are located at the top right side of the protocol screen and are used to edit, cut, copy, paste and delete method steps. To cut, copy, or delete a step, select the step with the mouse (for multiple step selection do a click-drag or Ctrl-click) and press the appropriate button. To paste steps into a method, select the step below the place where the new step(s) is to be added and press paste (see Table 7-12 for more detail).
- **File buttons**: The New Method, Edit Method and Browser toolbar buttons are located on the top left side of the protocol screen and are used to create new methods, edit a copy of the current method or to select an existing method (see Chapter 6.0 for more detail).

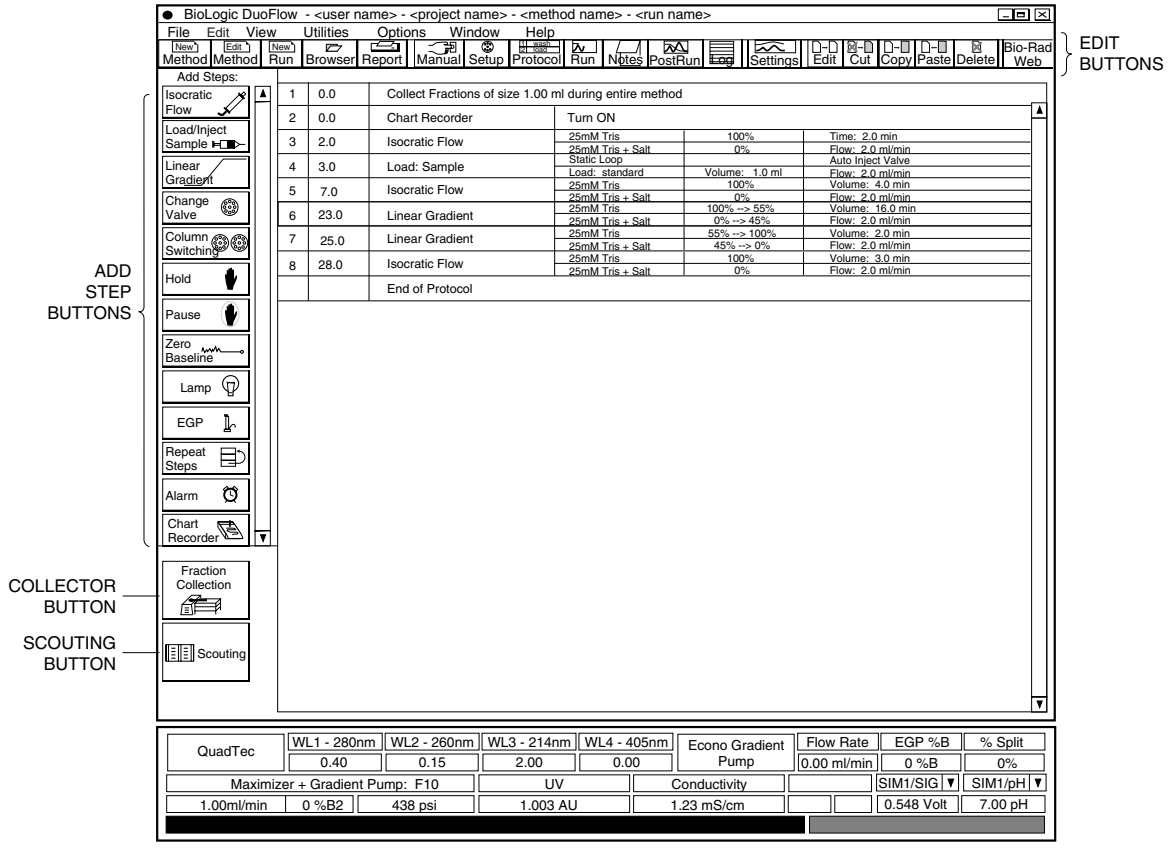
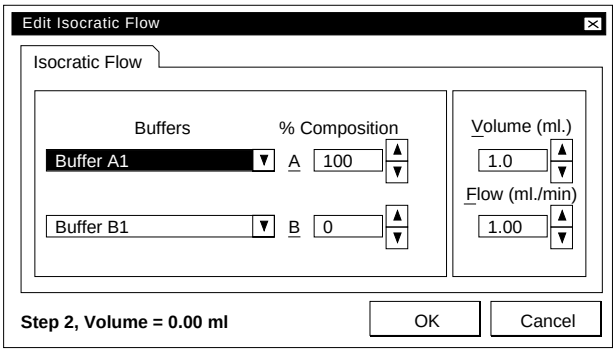
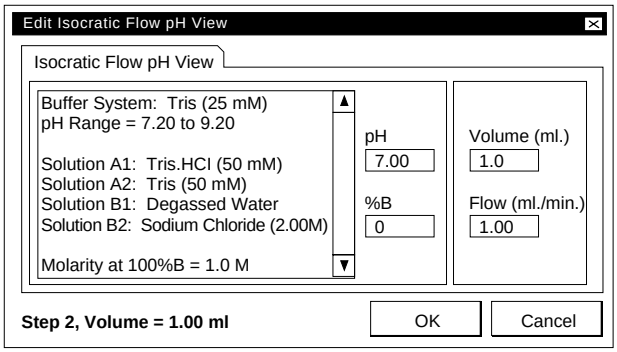


Figure 7-4. Protocol Screen

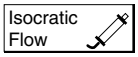
Table 7-3.
Isocratic Flow



Isocratic Flow Screen



Isocratic Flow Screen with Maximizer in Use



The Edit Isocratic Flow dialog is used to define the isocratic step buffer composition. The buffer composition is determined by the percentage of total buffer delivered through pumps A (%A) and B (%B).

- **Buffers**: Displays all the available buffers. The names displayed for Buffer A and Buffer B are the names assigned to the pump inlets or inlet valves in the device setup (see Chapter 7.2.2).
- **Composition**: Allows you to set the buffer composition for the step (e.g., 75% A and 25% B).

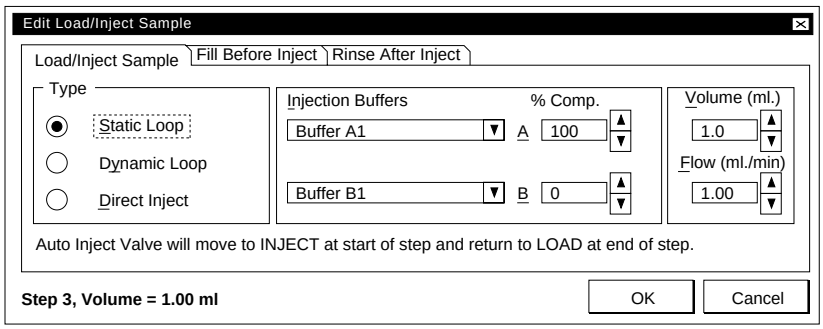
The **Edit Isocratic Flow pH View** dialog is used to define the isocratic step buffer composition when a Maximizer is connected to the system and Buffer Blending is turned on. The dialog box on the left lists the current buffer system, its pH range and the solutions assigned to each Maximizer valve inlet.

- **pH**: Allows you to enter the pH for the current step.
- **%B**: Allows you to set the buffer composition for the step as a percentage of the 1x salt concentration.

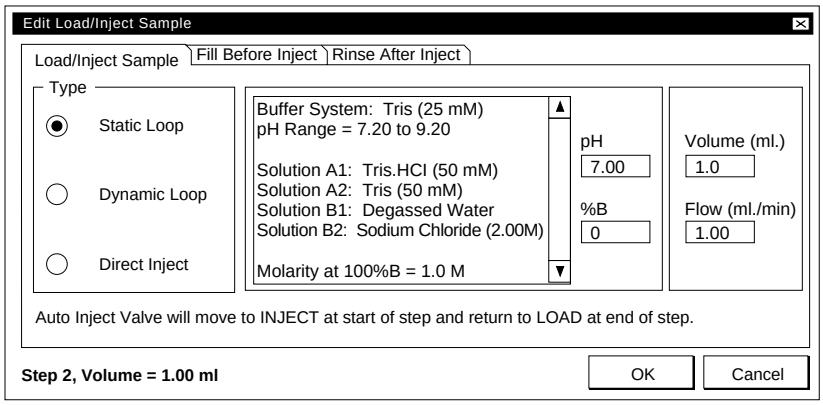
The following selections are available in both the **Edit Isocratic Flow** screen and the **Edit Isocratic Flow pH View** screen.

- **Volume/Time**: Allows you to choose the duration of the step.
- **Flow rate (ml/min)**: Allows you to choose the flow rate of the step.
- **OK**: Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel**: Cancels all input. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time** or **Volume**: Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-4.
Load/Inject Sample



Load/Inject Sample Screen



Load/Inject Sample Screen with a Maximizer in Use

Load/Inject Sample

The Edit Load/Inject Sample dialog is used to program sample injection. The appearance of this dialog and its associated tabs depend on the injection type selected and the devices configured in the device setup.

- **Injection Buffers**: Displays all the available buffers. The names displayed for Buffer A and Buffer B are the names assigned to the pump inlets or inlet valves in the device setup (see Chapter 7.2.2).
- **Composition**: Allows you to set the buffer composition used to push the sample onto the column (e.g., 75% A and 25% B).

When Buffer Blending is defined in the device setup the Buffer System, pH and %B fields are displayed.

- pH: Allows you to enter the pH for the current step. Displayed when Buffer Blending is included in the device setup.
- %B: Allows you to set the buffer composition for the step as a percentage of the 1x salt concentration. Displayed when Buffer Blending is included in the device setup.

Table 7-4. (continued)
Load/Inject Sample

The following selections are available in both dialogs.

- **Load/Inject Sample Tab**: Selects injection type. The AVR7-3 sample inject valve will move to the Inject position at the start of the step and return to the Load position at the end of the step.

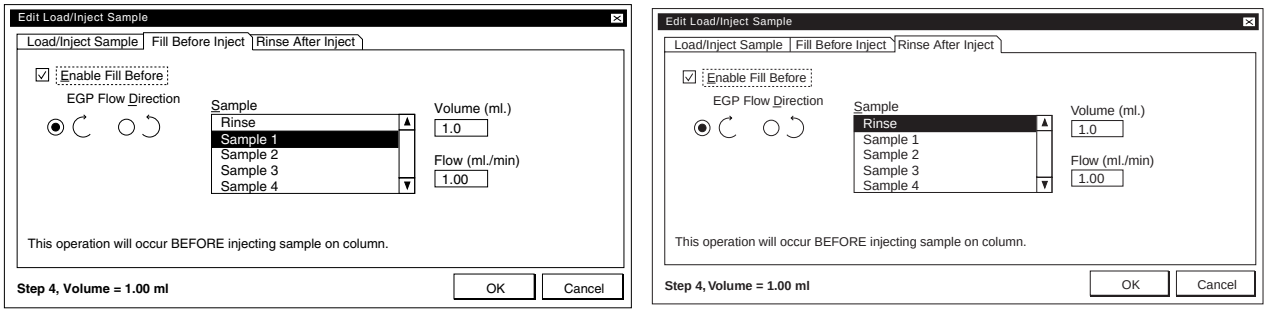
  Static Loop: Standard fixed volume loop for sample loading. Used with the AVR7-3 valve, sample can be loaded with a syringe through port 2, and the valve can rinse the sample. An auxiliary (AUX) pump and valve (such as the SVT3-2, SV5-4, or AVR9-8), may be used to fill the loop on the AVR7-3 prior to injection onto the column. Refer to Chapter 8 for discussion of how to select volume fill and rinse.

  Dynamic Loop: Uses Bio-Rad's DynaLoop or other sliding-piston sample loop. Both partial loop and full loop injections can be programmed. The dynamic loop can be filled manually through Inject valve port 2. An auxiliary (AUX) pump and valve (such as the SVT3-2, SV5-4, or AVR9-8), may be used to fill the loop on the AVR7-3 prior to injection onto the column. The Rinse function is not available. See Chapter 8 for DynaLoop loading instructions.

  Direct Inject: Allows direct injection of sample through either the Workstation pump (not available when Buffer Blending is turned on) or auxiliary load pump. Pre-pump valves (such as the SVT3-2, SV5-4, or AVR9-8) automate direct injection. Use of an auxiliary pump (such as the Bio-Rad Model EP-1 Econo pump or Econo Gradient Pump) allows direct injection of sample onto a low pressure column. Use of an auxiliary (AUX) pump and valve allows multiple direct injections onto a low pressure column. Refer to Chapter 8 for discussion of a direct inject applications.

- **Volume**: Allows you to specify the volume of the sample to be injected. The smallest volume selectable is 0.1 ml; the largest volume selectable is 9999 ml (for larger volumes, use another Load/Inject Sample step). Actual load volume is defined by the sample loop size.
- **Flow Rate**: Allows you to specify the flow rate for the sample injection step.
- **OK**: Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel**: Does ***not*** add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time** or **Volume**: Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

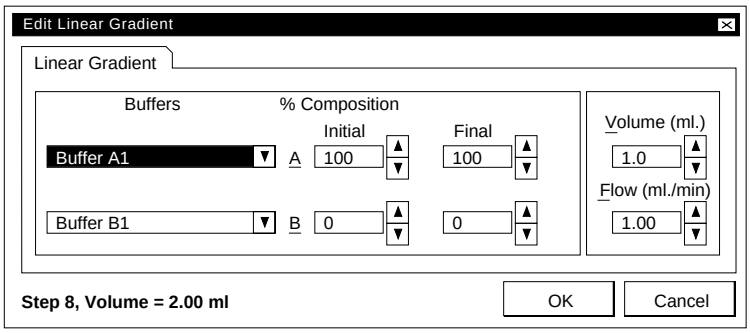
Table 7-4. (continued)
Load/Inject Sample



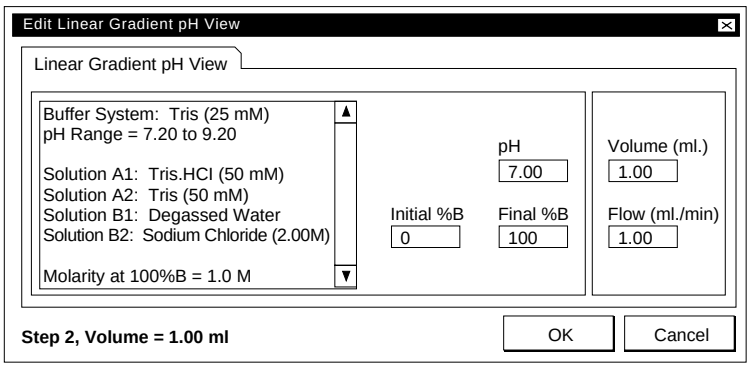
The **Fill Before Inject** and **Rinse After Inject** tabs are used to automatically fill a sample loop before an injection step or to rinse the loop after an injection has occurred. The Fill Before Inject tab is active for both static loop and dynamic loop injections if an auxiliary pump is defined in the device setup. The Rinse After Inject tab is active only for static loop injections if an auxiliary pump and auxiliary pump inlet valve are defined in the device setup.

- **Enable Fill Before**: Activates the Fill Before Inject controls.
- **Enable Rinse After**: Activates the Rinse After Inject controls.
- **Flow Direction**: Sets the flow direction for an Econo Gradient Pump. For an EP-1 pump or non Bio-Rad pump the flow direction is set at the pump.
- **Sample**: Used to select a sample or wash solution for use in the Load/Inject step. Active only if an Aux Pump Inlet valve has been defined for the method.
- **Flow**: Used to enter the auxiliary pump flow rate. Note that for an EP-1 pump or non Bio-Rad pump the flow rate must be set at the pump itself.
- **Volume**: Used to set the amount of solution loaded into the loop from the auxiliary pump.

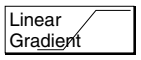
Table 7-5. Linear Gradient



Linear Gradient Screen



Linear Gradient Screen with a Maximizer in Buffer Blending Mode



Use the **Edit Linear Gradient** screen to deliver buffer gradients. The Linear Gradient screen sets the initial and final composition of the buffers and the period over which the change in composition is to occur. There is no limit to the number of gradient steps in a protocol. The following selections are available.

- **Buffers**: Allows you to choose two buffers from which to make a binary gradient. Drop-down menus display all buffers that are are assigned to the pump inlets or inlet valves in the device set up (see Chapter 7.2.2).
- **% Composition**: Allows you to choose initial and final values for the linear binary gradient. Notice that changing one value in the “Initial” column affects the other “initial” value. The “Final” column behaves identically.

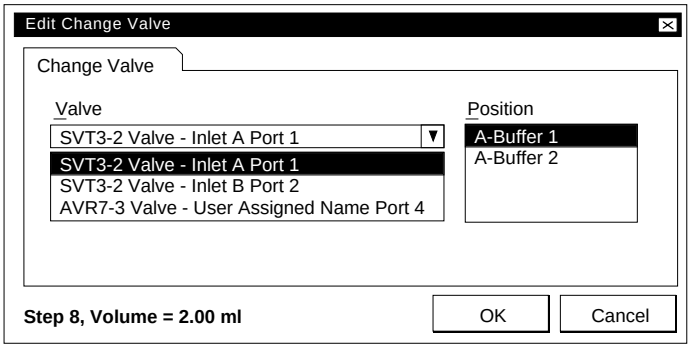
The **Edit Linear Gradient pH View** screen appears when you are using a Maximizer in Buffer Blending mode. This box sets the pH, initial and final salt concentration, and the period over which the change in composition is to occur. There is no limit to the number of gradient steps in a protocol. The following selections are available.

- **pH**: Allows you to enter the pH for the current step. The dialog box on the left-hand side lists the currently defined buffer system and its pH range as well as the solutions present at each inlet.
- **Initial %B and Final %B**: Allows you to set the initial and final salt concentration as a percentage of the maximium allowable concentration for a linear binary gradient.

The following selections are available in both screens.

- **Volume**: Allows you to choose the duration of this step.
- **Flow**: Allows you to choose the flow rate of this step.
- **OK**: Adds the step to the protocol. This is the same as pressing the Enter key on the keyboard.
- **Cancel**: Does not add the step to the protocol. This is the same as pressing the Esc key.
- **Step, Time** or **Volume**: Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-6.
Change Valve



Change Valve

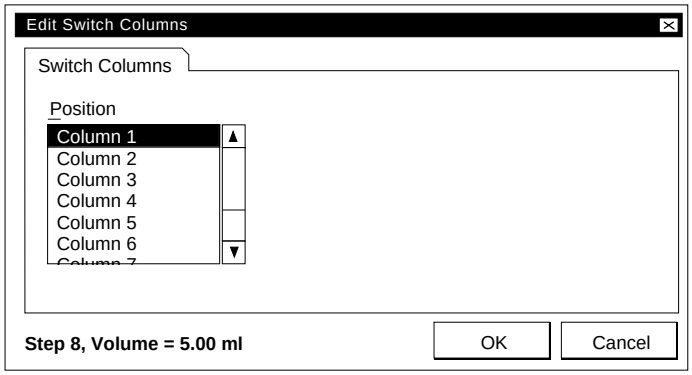
To select any valve and change its position.

- **Change Valve name and position**: These drop-down menus allow you to choose a valve and to make a change in valve position. Note that certain valve functions defined in the Setup screen (such as Fraction Collector Diverter, Aux Pump Inlet, Sample Inject, Inlet A, Inlet B) are “tied” to other protocol steps, so valve position changes will be made automatically. An example would be an AVR7-3 valve defined as a Sample Inject valve in the Setup screen, which is then tied to the Load/Inject Sample step in the Protocol screen.

  Valves assigned a “User-defined name” during Setup require the “Change Valve” step at the desired point in the protocol.

- **OK**: Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel**: Does ***not*** add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time** or **Volume**: Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-7.
Column Switching

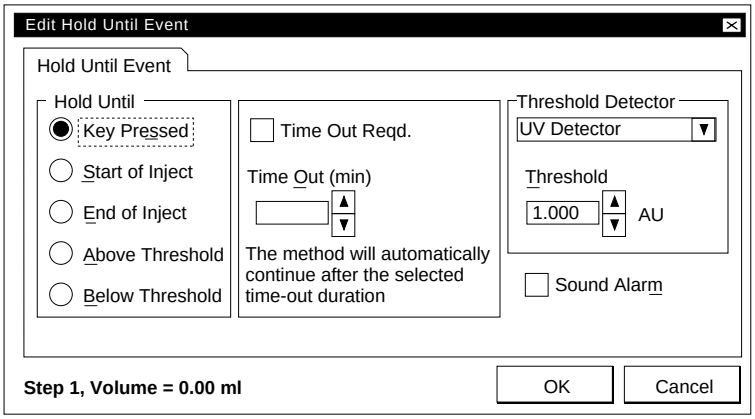


The DuoFlow system supports the use of more than one column during a run. (Chapter 9 provides two examples of column switching during a run.) Use this button to specify which column to load sample onto.

Column switching between as many as eight columns can be done with two AVR9-8 valves. A column switch valve is defined by pressing Column Switching in the device setup and assigning two AVR9-8 valves to the column switching valve. This identifies the two valves as installed and synchronizes the switching of the valves without further user input. Column switching permits identification of the electrical connection port of each valve and user naming for each column. Installation of the AVR9-8 valve in setup automatically activates Column Switching in the Protocol screen.

- **Position**: Select the column to switch to in a run.
- **OK**: Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel**: Does ***not*** add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time** or **Volume**: Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-8.
Hold



Hold

Inserts a Hold step into the method. The Hold step stops the progression of the method; the pumps continue pumping at the Hold step %B composition. The method time (or volume advances. When the run resumes, the current fraction collection condition is maintained. The hold condition is maintined until a specified activity occurs (e.g., “Hold until Start of Inject” or “Hold until Keypress”). For example, if the hold starts at time 2 (minutes), then the method resumes at time 2, no matter how long the Hold was in effect.

Hold Until and **Threshold Detector**: Choose one of five events to discontinue the hold:

- **Key Pressed**: Press the **F2** key on the keyboard to end the programmed hold.
- **Start of Inject**: The run will continue when the manually-controlled device (which must be connected to the Workstation AUX connector at pin 1, Inject) is moved to its desired position.
- **End of Inject**: The run will continue when the manually-controlled device (which must be connected to the Workstation AUX connector at pin 1, Inject) is returned to its original position.
- **Above Threshold**: The run will continue when the selected detector signal exceeds the specified threshold:

  Threshold Detector: Select the desired threshold detector absorbance, conductivity, RI, etc.

  Threshold: Specify a threshold value.
- **Below Threshold**: The run will continue when the selected detector signal falls below the specified threshold:

  Threshold Detector: Select the desired threshold detector absorbance, conductivity, RI, etc.

  Threshold: Specify a threshold value.
- **Time Out Reqd** and **Time Out (min)**: To hold for a specified length of time.
- **Sound Alarm**: When this box is checked, an alarm will sound at the beginning of the step to remind you that the system requires an action on your part to allow the method to advance.
- **OK**: Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel**: Does ***not*** add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time** or **Volume**: Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-9.
Miscellaneous

Button	Description
Repeat Steps	To repeat the highlighted step(s) a specified number of times. To highlight more than one step, hold down the **Ctrl** key while selecting steps with the mouse.
Pause	To pause the method at a specific step during the run. Pause stops the progression of the method and time, holds the %B composition, and stops the pumps. **Time Out Req'd** permits a pause time to be entered. The step will be paused for the specified length of time, after which it will automatically resume. An audible alarm may be programmed to sound when the step is paused and when it automatically resumes.
Alarm	To sound a 10 second audible alarm at the programmed time. The progress of the run will not be affected.
Zero Baseline	To zero the detector baseline at a programmed time.
Lamp	At system start-up, the default condition is Lamp ON. The lamp may be set to turn off at the end of a run. If the lamp is OFF, it takes about 20 minutes for the lamp to warm up prior to use.
EGP	To start/stop an Econo Gradient Pump at the programmed time for a user-defined task.
Chart Recorder	To turn the chart recorder on/off at the programmed time. Inserts a step that tells the chart recorder to start/stop the paper feed and lower/lift the pen.
Add Step V	To display additional steps so that they can be added to the method.

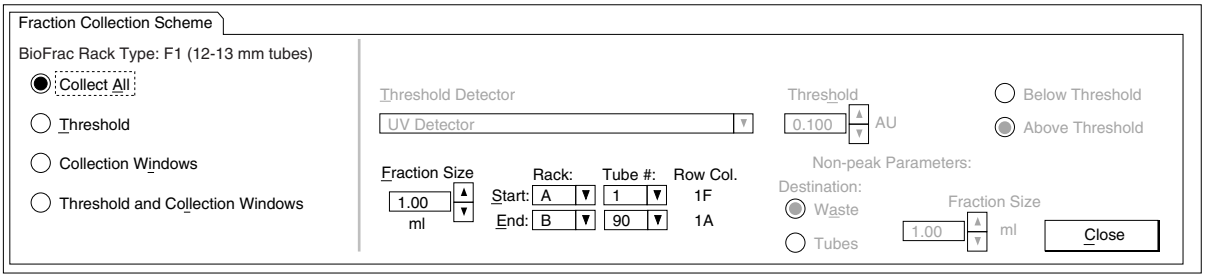
Table 7-10.
Fraction Collection

Button	Description: Collect All
Fraction Collection	To set up fraction collection. Fraction collection schemes are discussed below.

IMPORTANT NOTE:

The DuoFlow system is designed to control the BioFrac and the Model 2128 fraction collectors, which are the only collectors that provide a choice of racks and the ability to overlay fractions from consecutive runs.

The DuoFlow system will also accommodate the Bio-Rad Model 2110 fraction collector as well as generic collectors. To use a fraction collection scheme other than "Collect All" with these collectors, it is ***essential*** to assign a SVT3-2 valve as a "Fraction Collector Diverter" in the Setup screen. Rack choice and overlay features are ***not*** available with these collectors.

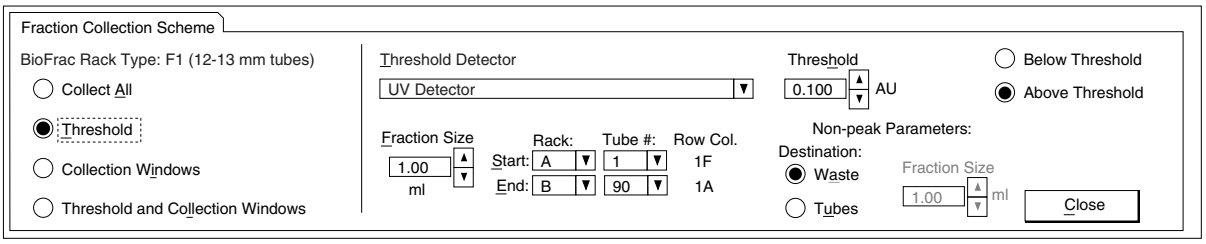


Collect All: The eluant from the entire run will be collected by the fraction collector.

- **Fraction Size**: Enter the fraction size as volume (ml).
- **Tubes Required** and **Number of Racks**: The displayed value is calculated based on the total volume divided by fraction size.
- **Start Tube** and **End Tube**: Identifies the first and last tubes to receive fractions. This applies only to the BioFrac and the Model 2128 fraction collector.
- **Start Rack** and **End Rack**: Identifies the first and last BioFrac rack to receive fractions.

Table 7-10. (continued)
Fraction Collection

Description: Threshold



Threshold: This method allows the flow to be sent to the collector if the detector signal of the eluant is above or below a certain signal level. **Note**: You can select any detection source to initiate threshold-based fraction collection. Non-peak eluant can be sent to waste or to the fraction collector.

- **Fraction Size**, **Start Tube**, **End Tube, Start Rack and End Rack**: See description for **Collect All** on page 7-20.
- **Threshold**: Enter the threshold value. Fractions will be collected whenever the detector output goes above or below the threshold level.
- **Above/below Threshold**: Used to select whether fractions are collected when the detector signal is above or below a designated Threshold.
- **Non-peak parameters Destination** and **Fraction Size**: Enter the non-peak parameters. If the buffer stream is diverted to Waste, there is no need to enter a fraction size. But if you want to collect non-peak material, you must enter a fraction size.

Table 7-10. (continued)
Fraction Collection

Description: Collection Windows

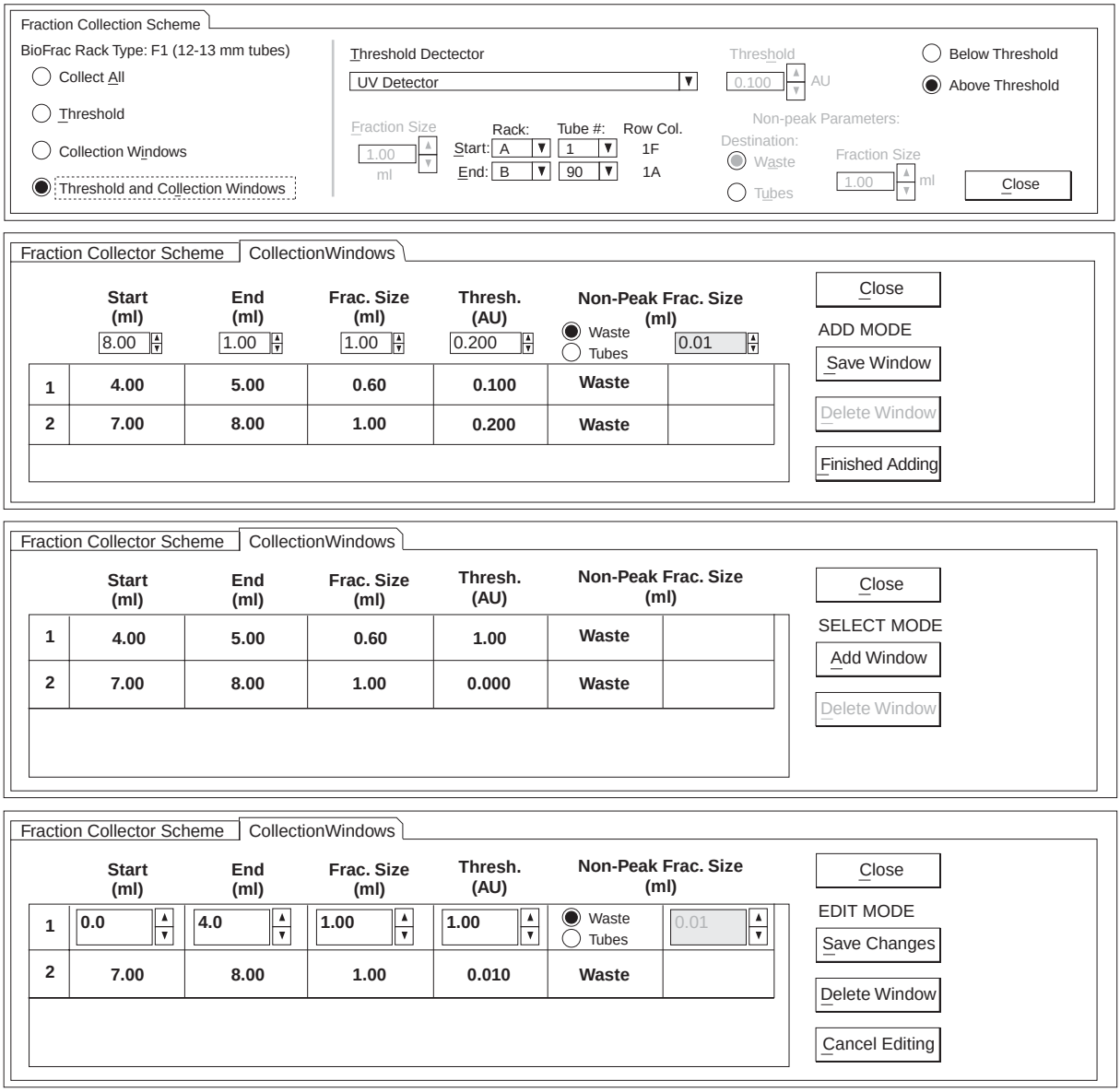
Collection Windows: This method specifies collection during specified parts of the run (Time or Volume). Each collection window can have a unique fraction size (ml).

Note: Collection windows programming should be done after all other steps have been written. When using Collection Windows, the BioFrac and Model 2128 will skip a tube between each window collection. For example, if the first window ends collection at 20 minutes and the second window starts collection starting at 21 minutes, then the fraction collector will leave an empty tube for the period between minutes 20 and 21.

- **Fraction Size**, **Tubes Required**, **Number of Racks**, **Start Tube**, **End Tube, Start Rack and End Rack**: See description for **Collect All** on page 7-20.
- **Add Mode**: Enter the desired values for **Start**, **End**, and **Frac Size**.
  a. Select the **Save Window** button and the values will appear in line 1.
  b. Enter the values for the next window and select **Save Window.** The values will appear in line 2.
  c. When the collection scheme is complete, select **Finished Adding,** and the Select mode will appear.
- **Select Mode**: This mode (not shown in illustration above) shows the following two buttons:
  Add Window: Inserts a window after the highlighted window.
  Delete Window: Deletes that window.

Table 7-10. (continued)
Fraction Collection

Description: Threshold and Collection Windows



Threshold and Collection Windows: Each Collection Window (discussed on page 7-22) can also contain a threshold value to further discriminate when eluant can be collected. For each collection window, non-peak eluent can be directed to waste or to the fraction collector. The Threshold and Time windows can be combined, so the system collects fractions only during the programmed Time windows and within the Time windows only when the detector signal is above or below the threshold.

When using the Collection Windows functions, the BioFrac and Model 2128 will skip a tube between each window collection. For example, if the first window ends collection at 20 minutes and the second window starts collection starting at 21 minutes, then the fraction collector will leave an empty tube for the period between minutes 20 and 21.

Table 7-10. (continued)
Fraction Collection

Description: Threshold and Collection Windows (continued)

- **Fraction Size**, **Start Tube, End Tube, Start Rack** and **End Rack**: See description for **Collect All** on page 7-20.
- **Above/below Threshold**: Used to select whether fractions are collected when the detector signal is above or below a designated Threshold.
- **Start (ml)**, **End (ml)**, **Fraction Size (ml), Thresh (AU),** and **Non-Peak Frac Size (ml)**: Each window is defined by these parameters. Use the scroll bar to the right of the window display to scroll through the list of windows. Enter the fraction size as volume (ml).
- **Add Mode**: Enter the desired values for **Start**, **End**, **Frac Size**, and **Thresh**.

  Select the **Save Window** button and the values will appear in line 1.

  To enter the values for the next window, select **Add Window** and enter the values. Select **Save Window** and the values will appear in line 2.

  When the collection scheme is complete, select **Finished Adding,** and the Select mode will appear.
- **Select Mode**: This mode shows the following two buttons:

  Add Window: Inserts a window after the highlighted window.

  Delete Window: Deletes that window.
- **Non-peak parameters Destination** and **Fraction Size**: Enter the non-peak parameters. When collecting non-peak material, you must enter a fraction size.

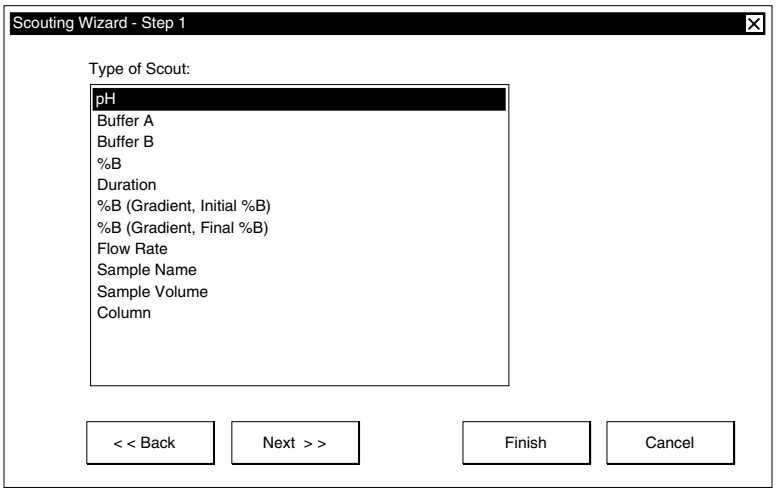
Table 7-11
Scouting

Scouting To setup a scout experiment for method optimization.

Scouting is a procedure used to systematically optimize the purification of a specific target molecule (i.e. protein). Molecules differ from one another in their charge, hydrophobicity, solubility, reactivity, substrate specificity and in their intermolecular interactions. A purification protocol that is satisfactory for one type of molecule may not work for a different molecule type. Several factors influence the quality of a purification procedure. Some of these factors include: buffer composition (pH, ionic strength, co-solutes), elution type (gradient slope and gradient duration), flow rate, column chemistry and sample composition. In principle, each of these can be adjusted to produce the most efficient and effective purification strategy for a molecule. In practice, only a few of these are generally tested due to time and cost considerations. By performing a series of automated scout runs, the time and resources required for protocol optimization can be significantly reduced.

Any method can be turned into a Scout Method using the Protocol Editor's Scouting Wizard. Methods used in scout experiments can be copied from an existing method, created from scratch or loaded from the Bio-Rad method templates. Before starting the Scouting Wizard, make sure the method setup and protocol are correct and that the step parameters are set to the values desired for the first scout run.

Scouting Wizard Step 1



This dialog is used to select the type of scout to be carried ran.

- **pH**: Used in Buffer Blending mode to find the optimal pH for a chromatography method.
- **Buffer A**: Used to automatically run an experiment using a variety of load, wash or elution buffer conditions through Inlet A.
- **Buffer B**: Used to automatically run an experiment using a variety of load, wash or elution buffer conditions through Inlet B.
- **%B**: Used to systematical alter the salt composition of the buffer mobile phase during isocratic flow and Load/Inject steps.
- **Duration**: Used to systematically vary the duration of isocratic flow, load/inject and linear gradient steps.

Table 7-11 (continued)
Scouting

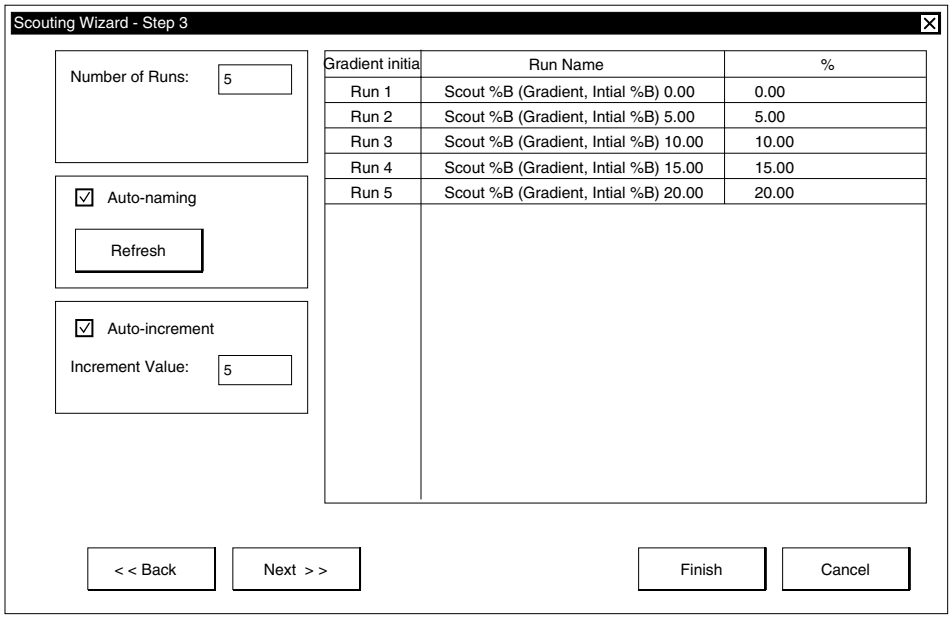
- **%B (Gradient, Initial %B)**: Used to systematically vary the initial buffer composition of a gradient step.
- **%B (Gradient, Final %B)**: Used to systematically vary the final buffer composition of a gradient step.
- **Flow Rate**: Used to optimize the flow rate for adsorption and elution steps.
- **Sample Name**: Used to inject samples that have been prepared under a variety of conditions
- **Sample Volume**: Used to find the optimal sample load volume.
- **Column**: Used to automatically test up to eight column types when an AVR9-8 column-switching valve is used. Alternatively, this method can be used similar to the Buffer A or, Buffer B options above, to simultaneously change Buffer A and Buffer B using the column switching valves.

Scouting Wizard Step 2

This dialog is used to select the protocol steps to be scouted. Steps that are incompatible with the current scout type are grayed out. Steps to be scouted are highlighted in green. Steps that have not been selected are highlighted in white. Steps to be concurrently scouted must all have the same initial value for the scouted parameter.

- **Select All**: Highlights the first step that is compatible with the selected scout type along with all steps that can be concurrently scouted with it. Concurrently scouted steps must each have the same initial value for the scout parameter.
- **Deselect All**: Deselects all protocol steps in scout setup.
- **Description/Parameters**: Displays the currently defined protocol and is used to select or deselect the steps to be scouted with a mouse.

Scouting Wizard Step 3



This dialog is used to set the number of runs to be performed, name each run and set the parameters for the scouted steps.

- **Number of runs**: Sets the number of runs to be performed as part of the scout experiment.
- **Auto-name**: When selected, this feature automatically generates run names based on the current Run 1 run name. Refresh updates the run names.
- **Auto-increment**: Automatically generates scout run parameters by incrementing the Run 1 scout parameter by the Increment Value. The scout Increment Value can be positive or negative. Note, you should create your protocol with the appropriate starting value before defining the scout.
- **Finish**: Saves the scout setup. Note, the protocol cannot be edited once a scout has been defined unless the scout step is deleted.

Table 7-11 (continued)
Scouting

Creating a Scout Experiment

Setting up a Scout Method

1. Connect and plumb all the required hardware components to the DuoFlow system.
2. Define the required devices in the device setup.
3. Prepare sufficient buffers for the number of runs to be performed
4. Prime the system and equilibrate the column(s) that will be used

Adding a Scout Step

1. Write a new method, copy an existing method or use a Bio-Rad method template. This method will be used for the first scout run. Make sure all parameters are set correctly and the necessary devices have been defined in the device setup. Once a Scout step has been added to a protocol, it cannot be modified unless the scout step is deleted.
2. Press the Scout button on the lower-left corner of the protocol screen to add a scout step to the protocol using the Scout Wizard.
3. In the Scout Wizard Step 1 dialog, select the parameter type to be scouted. As a general rule, those parameters that are expected to have the greatest impact on the chromatography results should be scouted first.
4. In the Scout Wizard Step 2 dialog, select the protocol step or steps to be scouted. Steps that cannot be scouted are grayed out, and steps selected for scouting are highlighted.
5. In the Scout Wizard Step 3 dialog, enter the number of runs, name the runs and for each run enter a value for the scouted parameter. The run name can be auto-generated or entered manually. Automatically generated run names use the Run 1 name as the base name. The value of the scout variable can be auto-generated or entered manually. Auto-generated values use the Run 1 value as the start value and increment the value by the Increment Value amount. The Increment Value can be a positive or negative number and is always defined as 1 when scouting valve positions (e.g. Buffer A, Buffer B, Sample Name and Column).

The following Toolbar menu options are available only when creating and editing a protocol.

Table 7-12.
Protocol Screen's Editing Toolbar

Button	Description
Edit buttons:	These buttons are available from the System menu.
Edit	To edit the currently highlighted step(s) in the protocol.
Cut	To cut the currently highlighted step(s) from the protocol. It places the step in the clipboard, which means it can be placed elsewhere by using the **Paste** button.
Copy	To copy the currently highlighted step(s) in the protocol.
Paste	To paste the cut or copied step(s) before the currently highlighted step.
Delete	To delete the currently highlighted step(s) from the protocol.

7.4 RUN SCREEN

The Run screen displays a run in progress. All data associated with the run are automatically saved. An example of a run in progress is shown in Figure 7-5 a and b, below. Table 7-13 discusses the buttons which may be used to control the run. In addition, if the EZLogic Integration software is installed, the **Integ**. toolbar button replaces the **Log** button.

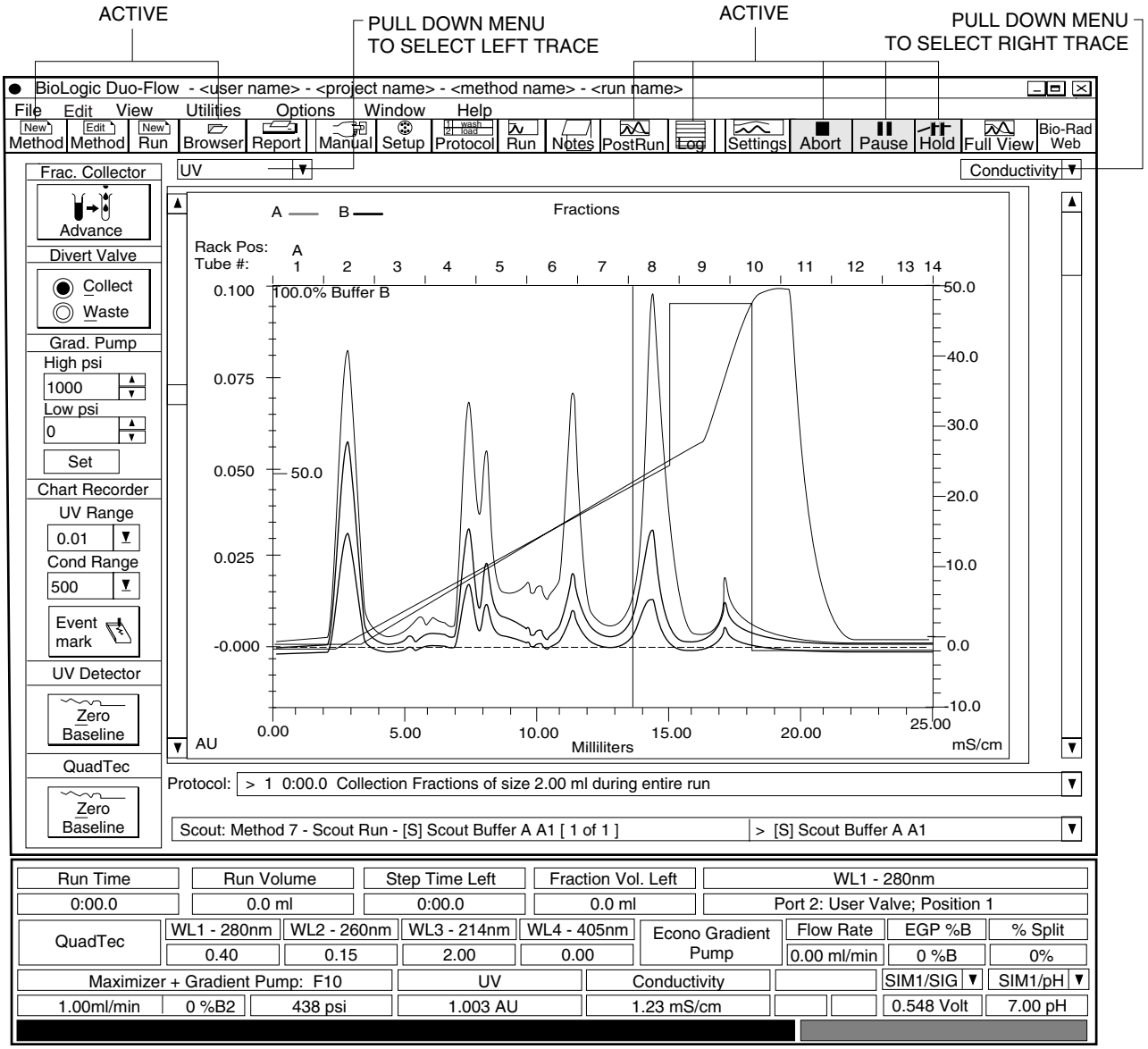


Figure 7-5a. Run Screen showing a Run in Progress with a Volume-based Chromatogram and "Rack and Tube#" fraction numbering

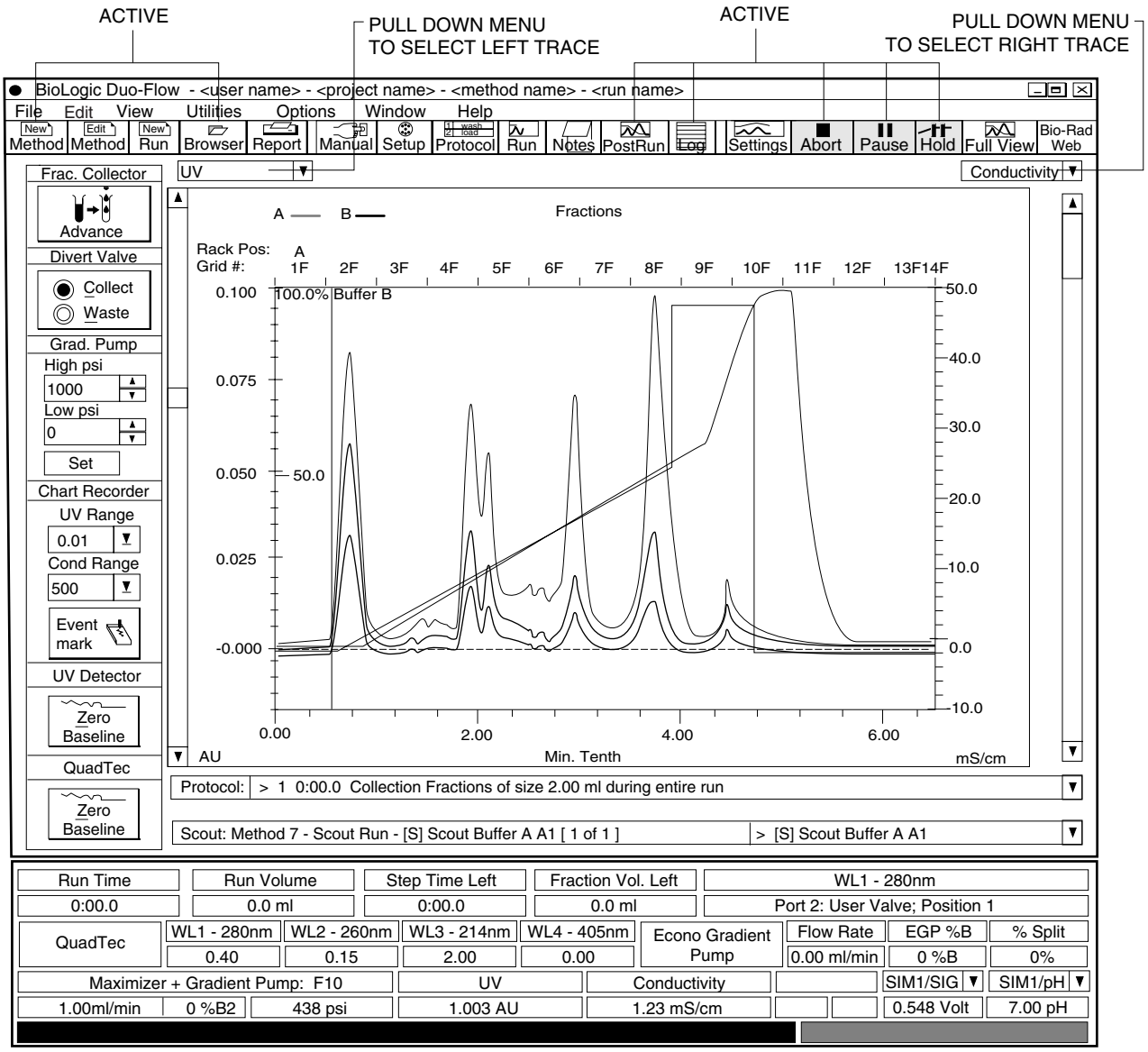


Figure 7-5b. Run Screen showing a Run in Progress with a Time-based Chromatogram and "Rack and Grid#" fraction numbering

Table 7-13.
Run Screen's Control

Button	Description
Frac. Collector Advance	Advances the fraction collector to the next tube. Pressing this button does not modify the method. The event is recorded in the Run Log.
Divert Valve Collect Waste	Immediately changes the position of the diverter valve. Pressing this button does not modify the method. The event is recorded in the Run Log.
Grad. Pump High psi 400 Low psi 20 Set	To set the pressure limits of the Workstation pumps. This is not part of a protocol; it is a system setting from the Manual screen. The method pauses and the pumps stop when the pressure goes above or below the set values.
Chart Recorder UV Range 0.2 Cond Range 500 Event mark	To set the **UV Detector** range for the chart recorder output. The settings are: 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 AUFS. To set the **Conductivity** range on the chart recorder output. The settings are: 500, 200, 100, 50, 20, 10, 5 mS/cm. To set an **Event Mark** on the chart recorder UV trace output only. It will not record event marks on the screen.
Zero Baseline	Use the **Zero Baseline** button to reset the UV or QuadTec detector absorbance value to zero. **Note:** Use care during a collection scheme which uses a **Threshold** value.
UV Conductivity	These drop-down menus, located on either side of the chromatogram, are used to select the active data trace to be scaled during or after a run. As each trace scale is selected, the scroll bars can be used to adjust the scale setting.
New Method Browser	To work offline while a run is in progress. (Refer to section 7.4.2, Working Offline.)
Notes Log	Notes displays the Run Notebook screen, which allows you to enter notes regarding the run. Log displays the Run Log screen, which provides information about the run and is non-editable. (Accessed from the view drop-down menu if EZ Logic has been installed).
Abort Pause Hold	To stop, pause, or hold a method in progress. (Refer to Figure 7-6.)
Settings	To select which traces to display in the chromatograms. (Refer to Table 5-7.)

7.4.1 Pausing/Stopping a Method in Progress

The Run screen provides three options for pausing or stopping a method in progress, as discussed in the figure below.

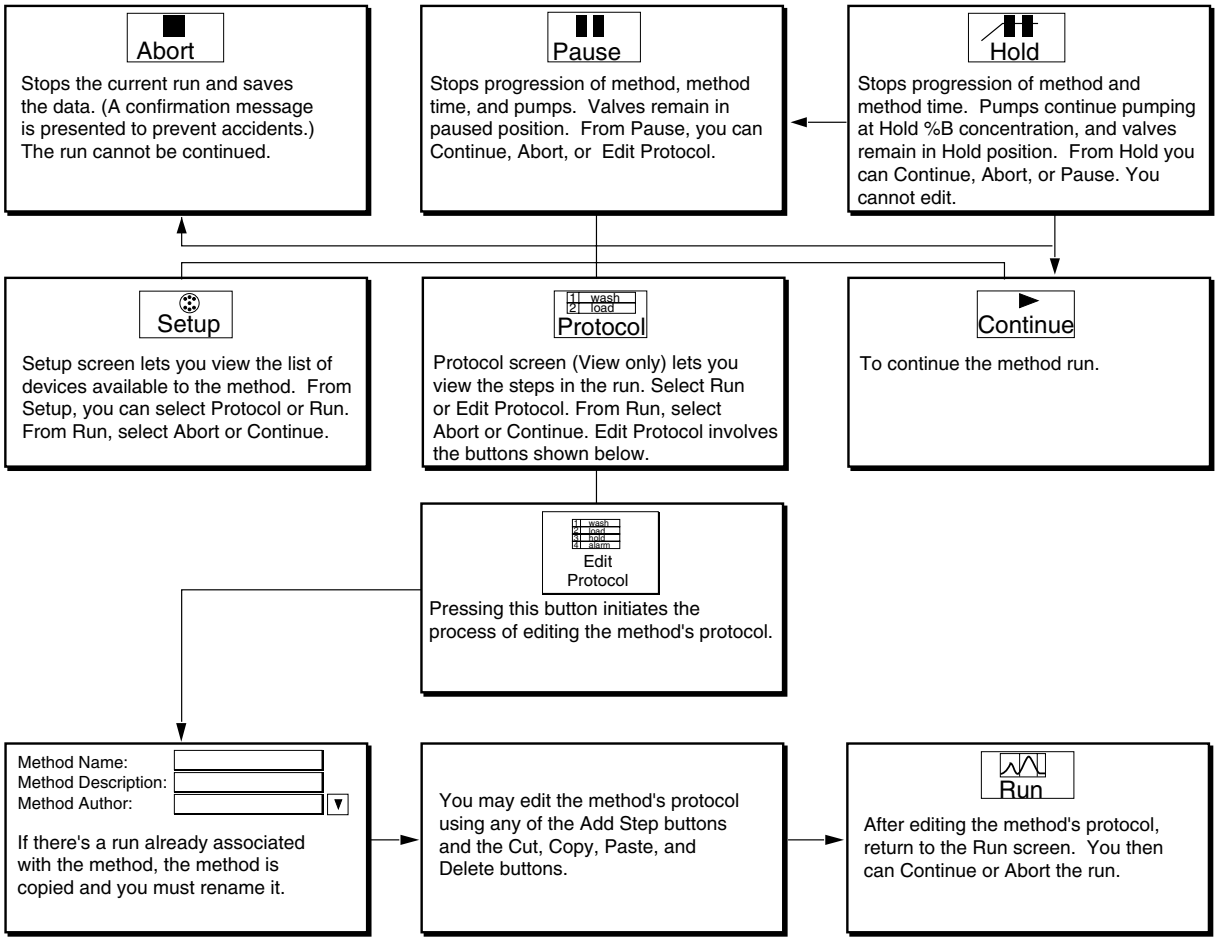


Figure 7-6. Run Screen's Abort, Pause, and Hold Buttons

7.4.2 Working Offline During a Run

The DuoFlow system software allows you to work offline, creating a new method or editing an existing method, while a run is in progress. The following actions can be performed:

- Write a new method
- Edit an existing method
- View results from a completed run
- Print a report from a completed run
- Integrate run data from a completed run (using the optional EZLogic software)
- Perform PostRun analysis on a completed run
- Export Data or Export Chromatogram Image of a completed run
- Access the HELP screen

These functions are not available while a run is in progress:

- PostRun analysis of the run in progress
- Integration of the data from the run in progress
- Copy Out data
- Copy In data
- Initiation of a new run
- Utilities functions, including calibrating pH probe, gradient pumps, conductivity
- Manual mode functions

During a run, the browser toolbar button is used to activate the offline window:

The online and offline windows are distinguished as follows:

- Online: The BioLogic DuoFlow icon in the upper left of the screen is green, and the title bar does not indicate online.
- Offline: The BioLogic DuoFlow icon in the upper left of the screen is yellow, and the title bar indicates offline.

To change between the online and offline windows, you can use any of the following:

- Windows drop-down menu: The online and offline windows are listed.
- Task bar: The Windows® taskbar lists the BioLogic DuoFlow windows.
- Alt-Tab: Simultaneously hold down these two keys to list the open windows.

7.4.3 Editing A Method During a Run

During a run, you may find you want to change some of the run parameters. The figure below shows how this is handled by the system and some of the restrictions that apply.

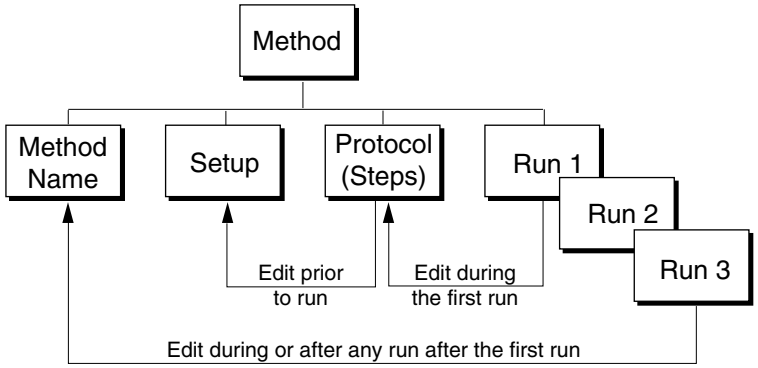


Figure 7-7. Editing during a Run

- During the first run of a method, you can **pause** the method and return to the Protocol screen to edit steps that have not already been started. The method name will not change.
- Any run after the first can be paused and then edited, except runs in queue or scout which cannot be edited. You will first be asked to rename the method (or accept the default new name) when the **Edit Protocol** button is clicked. This is to ensure the integrity of the database in terms of which method protocol a particular run is tied to.

  For example, assume you make three runs under a method named “Ion-exchange UNO Q1.” During the fourth run, you may decide to pause the run, and enter the Protocol screen to increase the length of the salt gradient. In this case, you will first be prompted to rename the method (e.g., Ion-exchange UNO Q1 Rev. 1) before proceeding with the edit.

  Upon completion of the run, the database of methods and runs will show three runs associated with the method “Ion-exchange UNO Q1” and one run associated with the method “Ion-exchange UNO Q1 Rev. 1.”
- A fraction collection scheme is editable, but it cannot be added to a protocol once a run has been started. In such cases, you should abort the run and select **Edit the Method**.
- A currently programmed fraction collection scheme may be edited during a run, but note that changing the fraction size of a “Collect All” scheme will first require selection of “Collection Windows.” This is because the editing process effectively turns the initial “Collect All” scheme into the “first” collection window.

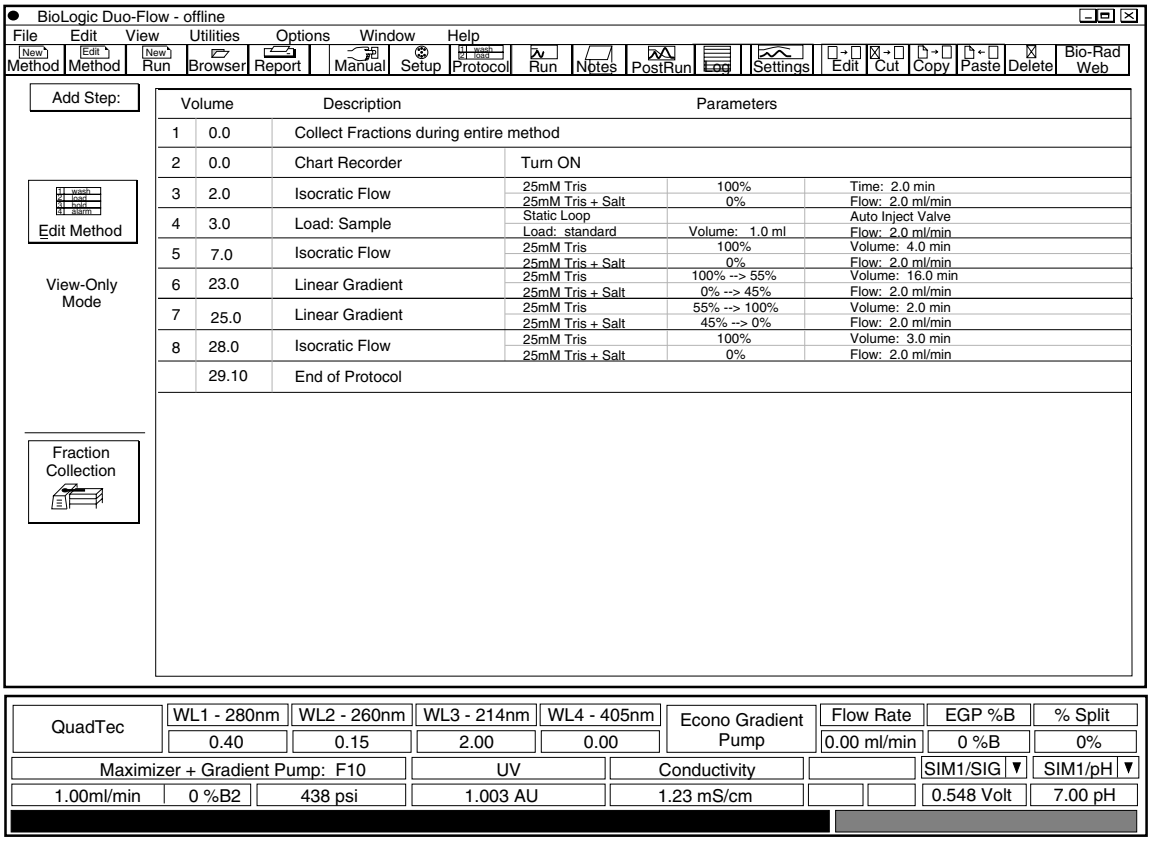


Figure 7-8. Protocol Screen during a Run

7.4.4 Run Notebook Screen

Use the Run Notebook screen to maintain any information you want regarding the run. It contains fields for description of the sample, the column, the operator, the buffer(s), flow rates, gradients, chart speed, fraction size, and any other information you might want to enter.

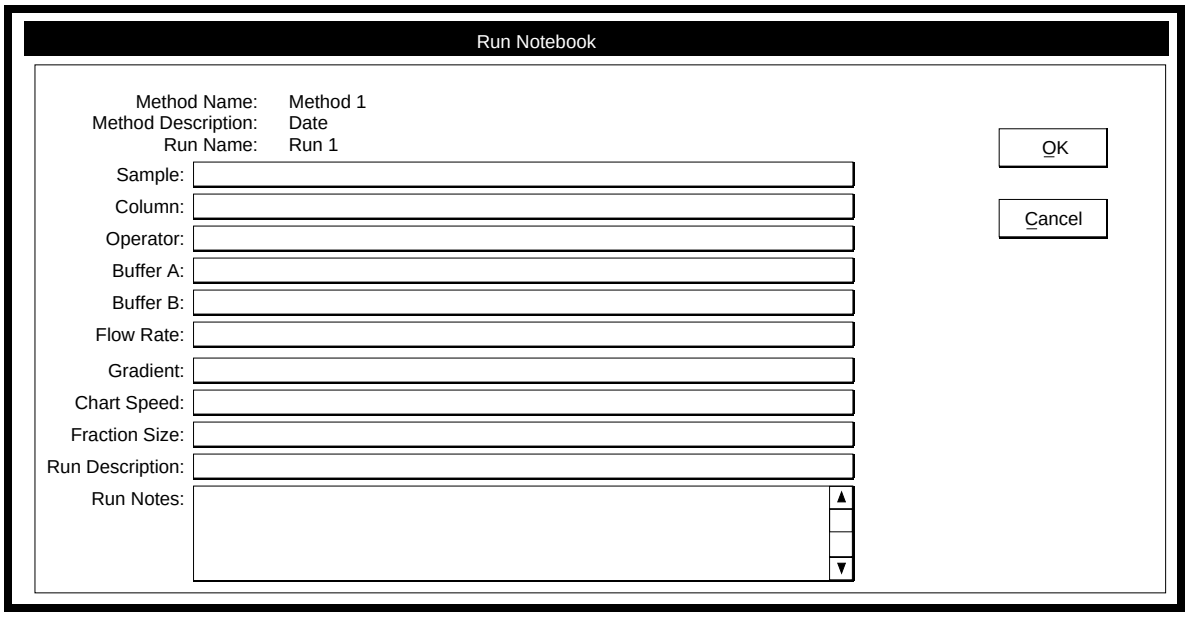


Figure 7-9. Run Notebook Screen

7.4.5 Run Log Screen

The Run Log screen details the time and order of the execution of each step, error, and/or event of the run. This information cannot be edited. It is possible, although not recommended, to turn off the Run Log by deselecting its checkbox in the Edit User Preferences window, available from the **Options** menu.

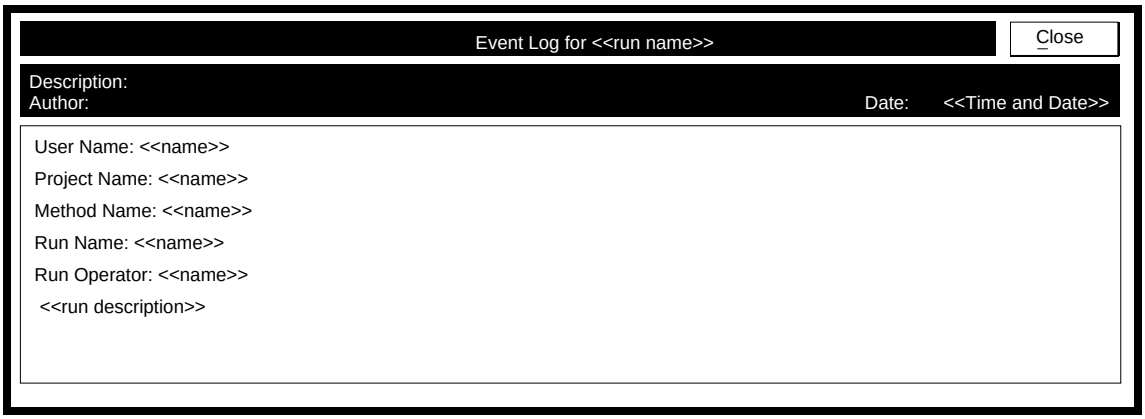


Figure 7-10. Run Log Screen

7.5 POST RUN Screen

The Post Run screen enables peak annotation (“tagging”) and data export. To enter the Post Run screen, open a run file in the Browser screen, or when a run is finished choose **PostRun** from the toolbar. Two chromatograms are viewed: the main chromatogram in the lower large window and the reference chromatogram in the upper right window. In the upper reference chromatogram, use the vertical scrollbar to adjust the scale.

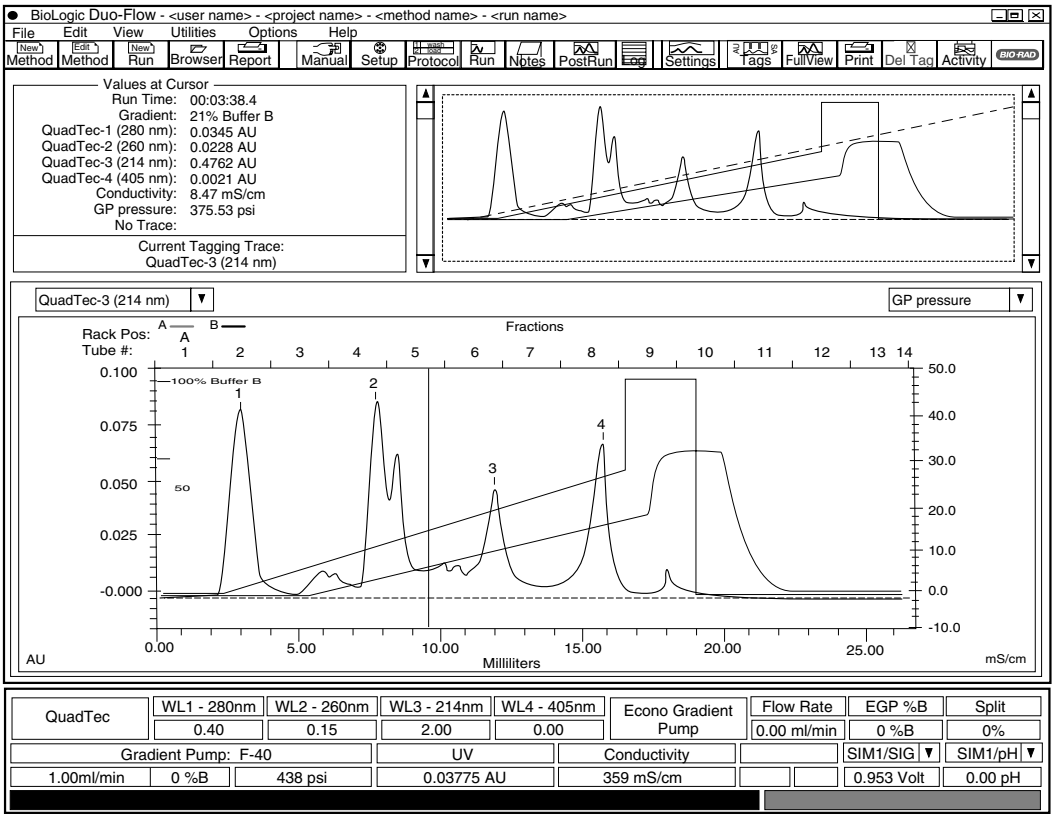


Figure 7-11a. Post Run Screen showing a Volume-based Chromatogram and "Rack and Tube#" fraction numbering

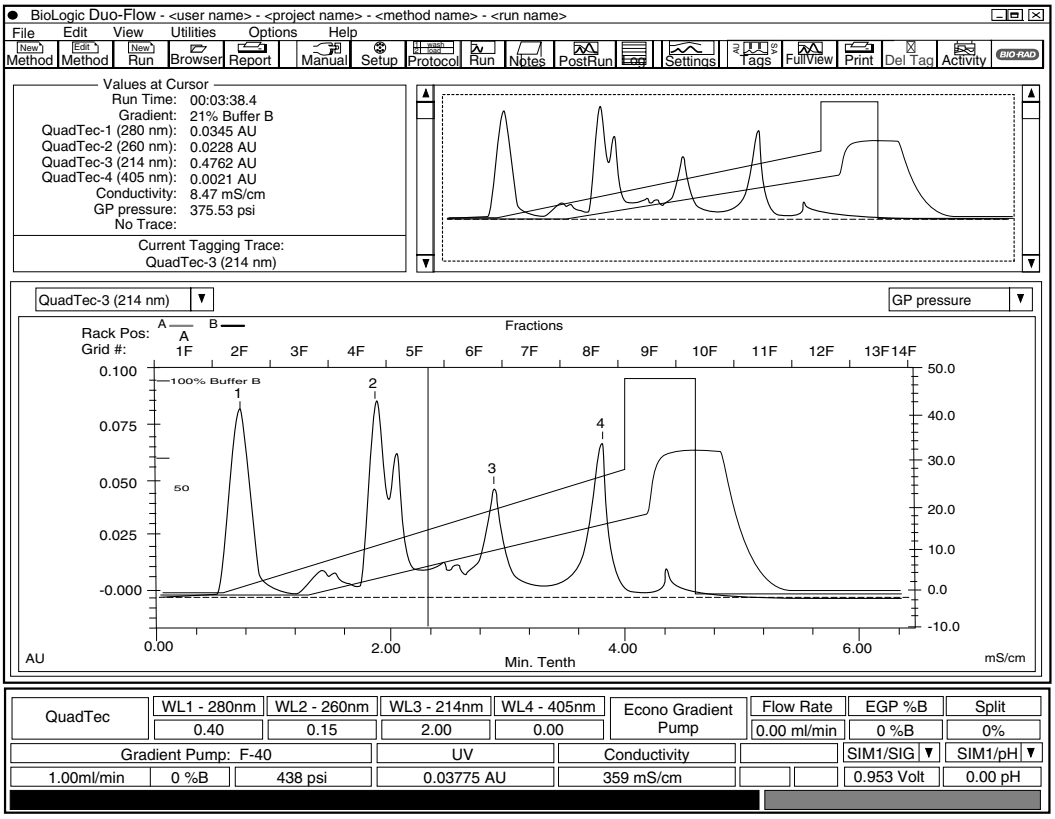


Figure 7-11b. Post-Run Screen showing a Time-based Chromatogram and "Rack and Grid#" fraction numbering

7.5.1 Resizing

The main chromatogram shows the current “Zoom” region. To resize the image (or “zoom in” to a particular region of the chromatogram), either change the axes scale in the reference chromatogram, or use the “rubber band” controls on either chromatogram. To use the rubber band controls, click and hold the left mouse button. Drag it across the chromatogram region. Release the mouse button and the main chromatogram will be resized. Click on the **Full View** button in the toolbar to return to normal view.

7.5.2 Chromatogram Information (Values at Cursor)

The top left corner of the PostRun screen contains information about the chromatogram. The Values at Cursor window in the upper left corner will list up to eight detector traces depending on the devices selected in the runs device setup. The position of the vertical bar within the main chromatogram dictates the displayed information. Run time, absorbance (AU), conductivity (mS/cm), gradient progression (%B), and the activity trace values are displayed for each trace at the position of the vertical bar. To view the vertical bar, place the cursor inside the main chromatogram.

7.5.3 Annotating (“tagging”) the Chromatogram

Tags can be placed on any trace. To assign tags to a trace, and to view all assigned tags and their data, use the Post Run Tags window, shown below. To display the Post Run Tags window, click once on the **Tags** button in the toolbar or from the Edit drop down menu, select **Tag and Trace Options.**

In the Post Run Tags window, select a trace by clicking on an Active Trace radio button. Trace visibility and tag visibility can also be selected from this box. Tag styles are specified in the upper right box. Peaks can be annotated with a sequential tag number (1,2...), a user-defined name (user tag name), with the trace value, or the run time.

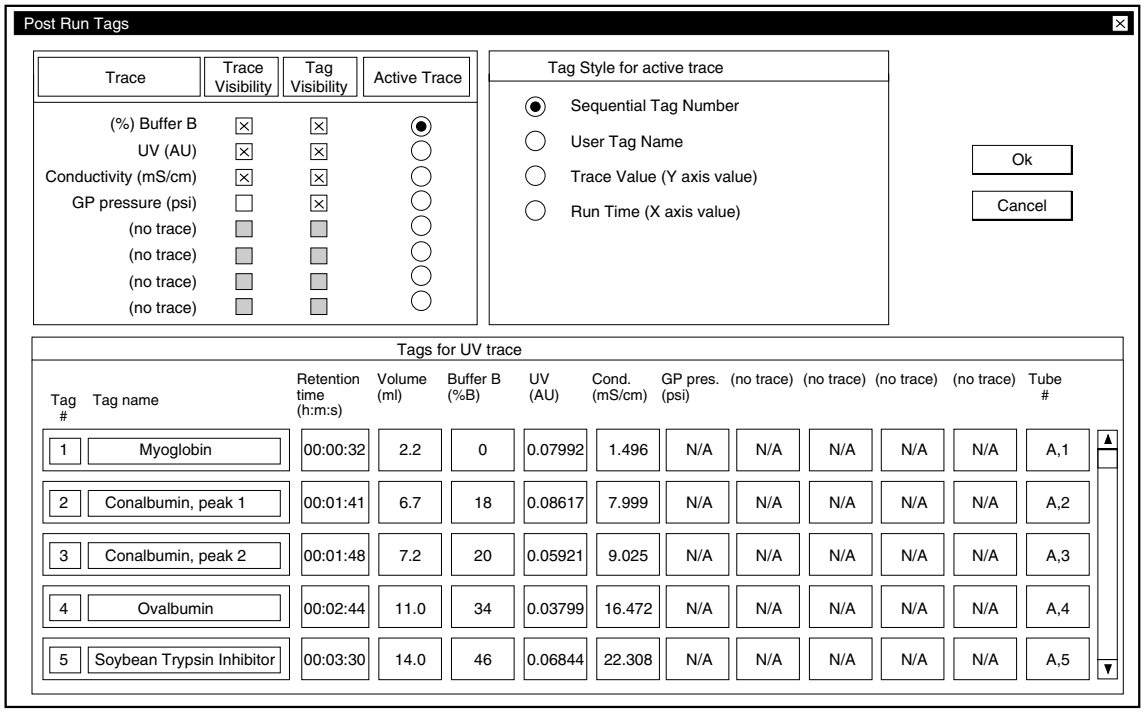


Figure 7-12. Post Run Tags for UV Detector
(Note: Additional Traces will be shown when the QuadTec Detector is being used.)

To change a tag name in the Post Run screen chromatogram, move the cursor over the tag and note that the cursor changes. Double click on the tag and in the window that appears, enter the tag name. To deselect a tag, highlight the undesired tag and press DelTag from the button bar. To remove all tags, select “Delete all tags” from the **Edit** drop-down menu.

7.5.4 Entering Activity Data

The Activity Trace Editor permits data collected by a separate offline method to be included with data collected by the DuoFlow method. For example, if fractions collected by the DuoFlow are also analyzed by an ELISA method or for radioactivity, CPM, or DPM, the data collected by these assays can be entered into the Activity Trace Editor and a trace will appear on screen reflecting the activity for each fraction.

1. To enter activity data: Select the run for which you wish to enter the post run activity data from the Browser screen.
2. Press the **Activity** toolbar button. A box will appear listing each fraction collected in the opened run. (See Figure 7-13.)
3. Enter activity values for each fraction and enter the desired units.

   Check the Show Activity Trace box to display the data in the Post Run screen.
4. The activity values entered will appear in the chromatogram information screen and as a trace in the chromatogram. (See Figure 7-14.)

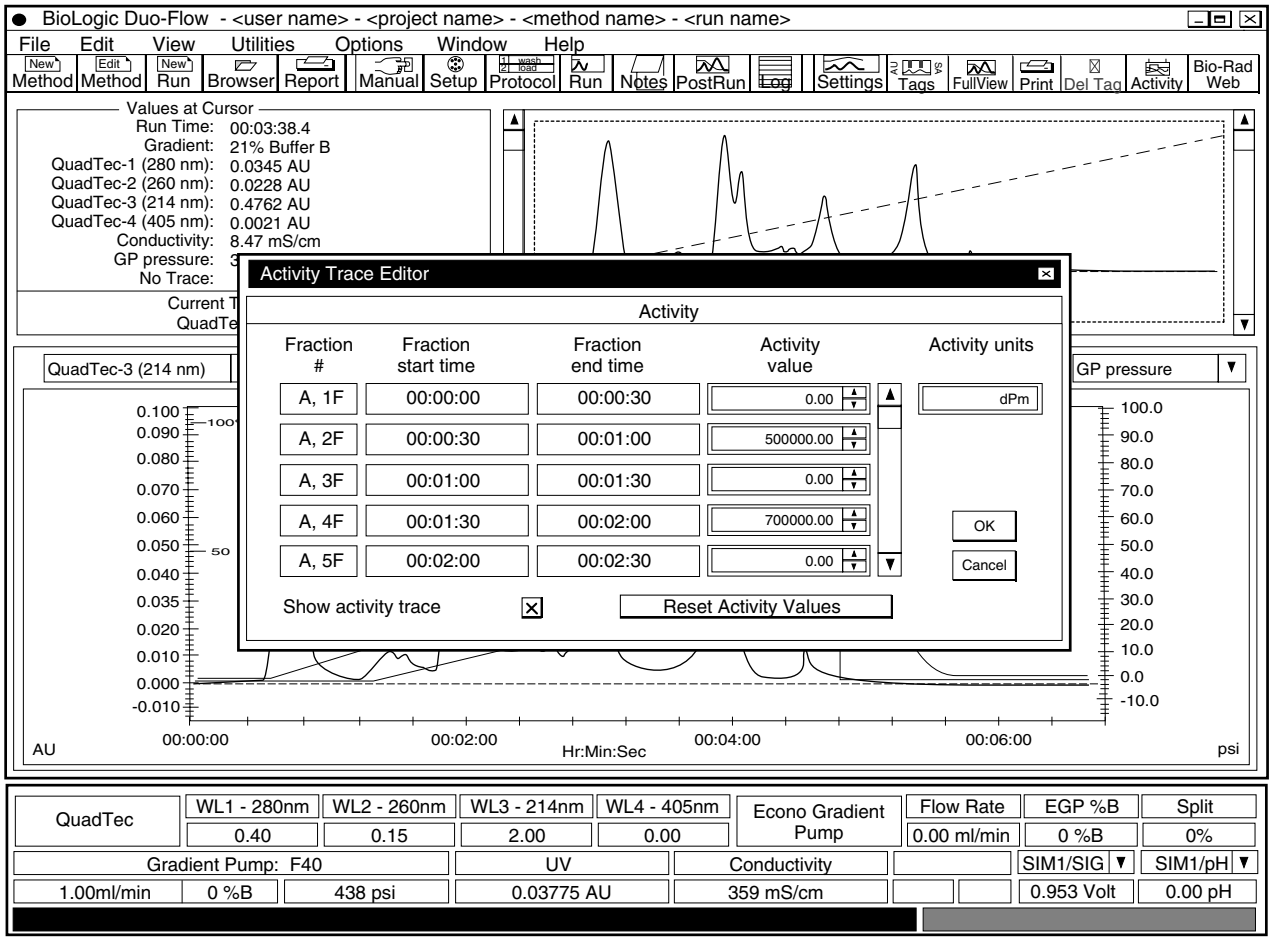


Figure 7-13. Activity Trace Editor

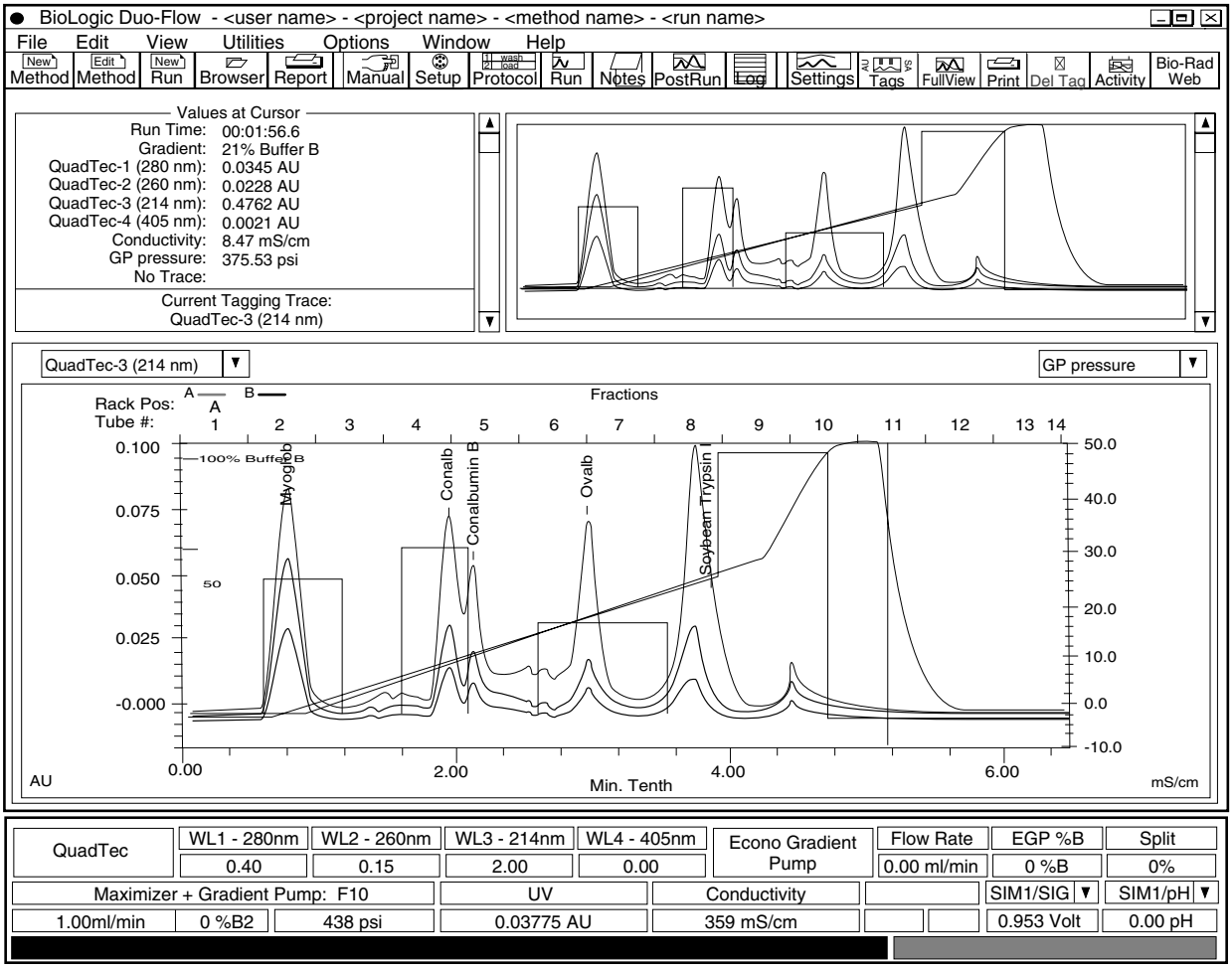


Figure 7-14. Activity Trace

7.5.5 Exporting Chromatogram Data to other Software Applications

To export data, go to the Post Run screen of the desired run. Select **Export Data** from the File drop-down menu (See Figure 7-15, Export Data Setup screen). From this screen, choose the run time and the data trace(s) that will be exported. Although data is collected at a rate of five points per second, it can be exported at a user-defined rate. Data is exported in an ASCII.TXT file format. The file can be imported into Excel as comma delimited data.

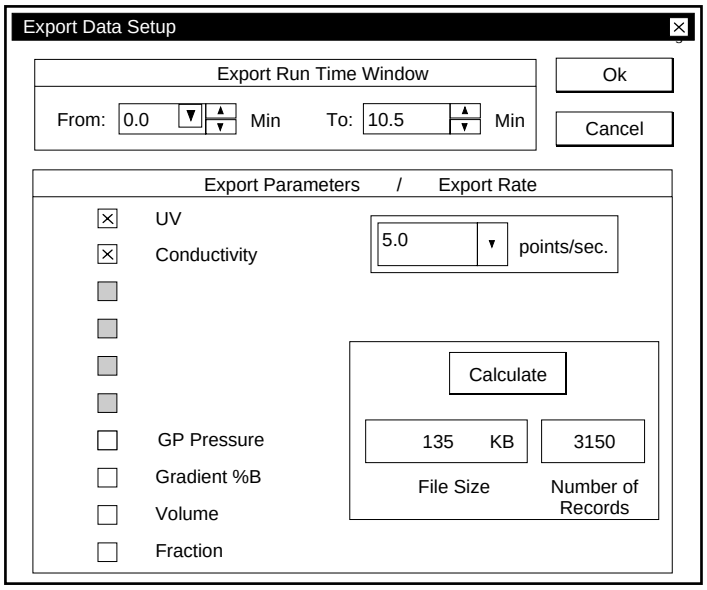


Figure 7-15. Export Data Setup Screen

7.5.6 Exporting Chromatogram Images

Chromatographic images can be exported into other applications, such as word processing programs. Before transferring an image, modify the chromatogram by selecting the desired traces and set the scale and zoom region in the PostRun screen. This should be done now since changes cannot be made to the file once it is transferred out of the DuoFlow program. The image displayed in the main chromatogram will be exported.

Select **Export Chromatogram Image** from the File drop-down menu (see Figure 7-16). The image is exported in a Windows Meta File (*.wmf) format which can be transferd into most Windows-based applications.

Alternatively, a chromatogram can be copied to the Windows clipboard and pasted into another Windows program. Choose **Copy zoom Chromatogram to clipboard** from the Edit drop-down menu.

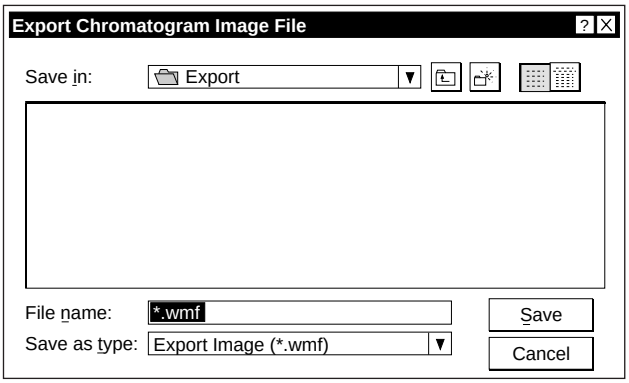


Figure 7-16. Exporting a Chromatographic Image

8.0 SAMPLE LOADING

The BioLogic DuoFlow system is capable of running both simple and complex experiments types. This Section describes how the optional hardware components can be used to incorporate a variety of advanced features into a method.

The BioLogic DuoFlow system supports several methods for sample loading. Typically a sample is loaded through a static fixed volume loop as described in Chapter 4. However, when automation or large volume sample injection is required, the following options are available:

- Automatic loop fill and rinse using an auxiliary pump inlet valve and an auxiliary pump
- Direct sample loading onto low-pressure columns using an auxiliary pump such as the Econo Gradient Pump (EGP) or EP-1 Econo pump.
- Direct sample loading through the Workstation pump
- Sample loading using the DynaLoop sliding-piston loop

8.1 AUTOMATIC LOOP FILL AND RINSE

With the addition of an Econo Gradient Pump, EP-1 Econo pump or other compatible pump as an auxiliary load pump along with an auxiliary pump inlet valve (AVR9-8), the system can load up to 7 samples sequentially into a loop. One of the Aux pump inlet valve ports must be assigned to a rinse solution. Similarly, an SVT3-2 or SV5-4 Aux pump inlet valve may be used to load 1 samples or 3 sample, respectively.

System Setup

1. Connect an auxiliary pump to the system as described in Section 3.9.1 (EP-1 or non-Bio-Rad pump) or 3.9.2 (Econo Gradient Pump).
2. Plumb the Aux Pump Inlet valve and Aux Pump to port 2 of the AVR7-3 Sample Inject valve as shown in Figure 8-1.
3. In the device setup screen, add an Aux Pump to the setup and define it as a load pump. Define non-Bio-Rad pumps as an EP-1 load pump.
4. In the device setup screen, add an AVR9-8 (or SV5-4 or SVT3-2) valve. Define the valve as an Aux Pump Inlet valve and name each position. One position (usually position 1) should be defined as a "Rinse" solution. The rinse solution is used to clean the sample loop and sample inlet lines between injections. Note that some sample will be lost in the process of pulling sample from a remote beaker. Be sure to factor in the solution volume required to fill all the buffer lines leading up to the top of the column when programming the injection step.
5. When using a low-pressure column, such as an Econo-Pac cartridge, remove the 40-psi backpressure regulator from the post-column position and place it between the Workstation pump and the mixer.

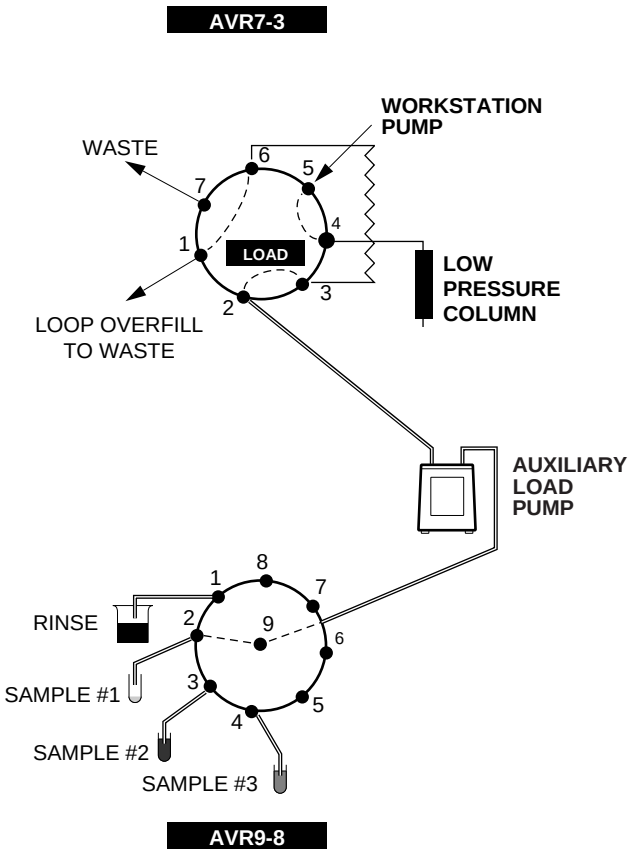


Figure 8-1. Multiple Sample Loading with an Auxiliary Load Pump and an AVR9-8 Valve

Writing an Automatic Loop Fill and Rinse Protocol

The protocol may be written as a single protocol containing back-to-back experiments or may be written as a series of queued runs. The latter option generally produces a simpler protocol that may be copied into a queue (once for each experiment to be run). The Load/Inject step can then be edited for each method in the queue so that the appropriate sample is loaded.

Several methods that include the Automatic Loop Fill and Rinse feature have been included in the BioLogic DuoFlow Method Templates (see Section 6.2 and the online help for more information). This feature may be added to any protocol using a static loop.

1. In the protocol load/inject step select Static Loop as the injection type.
2. In the Fill Before Inject tab, check the Enable Fill Before box, select the sample, and set the sample volume and flow rate. Note that if an EP-1 or non-Bio-Rad pump is used, the flow rate must be set both in this dialog and at the pump. Note that the fill time must be less than the duration of the preceeding step.
3. If the sample loop and tubing are to be rinsed between sample injections select the Rinse After Inject tab, check the Enable Rinse After box, select the "Rinse" buffer, and set the rinse volume and flow rate. Note, if an EP-1 or non-Bio-Rad pump is used the flow rate must be set both in this dialog and at the pump.

8.2 AUX PUMP DIRECT INJECT

With the addition of an Econo Gradient Pump, EP-1 Econo pump or other compatible pump to the DuoFlow system, samples can be loaded directly onto low-pressure columns. Up to 7 samples can be loaded sequentially, if an Aux pump inlet valve (AVR9-8) is connected to the system and defined in the setup. One of the Aux pump inlet valve ports must be assigned to a rinse solution. Similarly, an SVT3-2 or SV5-4 Aux

pump inlet valve may be used to load 1 samples or 3 samples, respectively.

System Setup

1. Connect an auxiliary pump to the system as described in Section 3.9.1 (EP-1 or non-Bio-Rad pump) or 3.9.2 (Econo Gradient Pump).
2. Plumb the Aux Pump to port 3 of the AVR7-3 Sample Inject valve as shown in Figure 8-2. If an auxiliary pump inlet valve is connected to the system it should be plumbed between the load pump and the AVR7-3 Sample Inject valve.
3. In the device setup screen, add an Aux Pump to the setup and define it as a load pump. Define non-Bio-Rad pumps as an EP-1 load pump.
4. (Optional) If an Aux Pump Inlet valve is used, add the valve to the device setup, define the valve as an Aux Pump Inlet valve and name each position. One position (usually position 1) should be defined as a "Rinse" solution. The rinse solution is used to clean the sample loop between injections. Note that some sample will be lost in the process of pulling sample from a remote beaker. Be sure to factor in the solution volume required to fill all the buffer lines leading up to the top of the column when programming the injection step.
5. Remove the 40-psi backpressure regulator from the post-column position and place it between the Workstation pump and the mixer. This allows low-pressure peristaltic pumps to push sample through the column while at the same time will help the check valve to seat properly and ensure pump flow performance. In addition, low-pressure columns may burst if the backpressure regulator is placed after the column.
6. Plumb the Aux Pump to port 3 of the AVR7-3 Sample Inject valve as shown in Figure 8-2.
7. (Optional) If an Aux Pump Inlet valve is used, connect it to the Aux pump inlet.

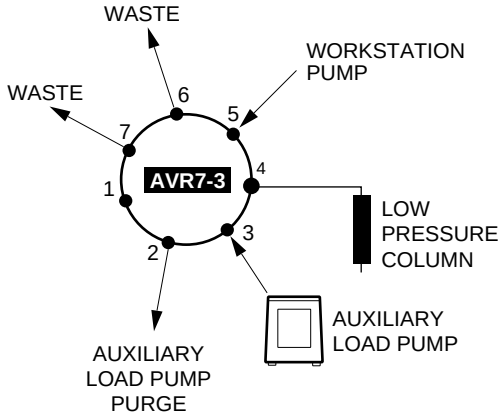


Figure 8-2. Plumbing an AVR7-3 Inject Valve with an Auxiliary Load Pump

Writing the Aux Pump Direct Inject Protocol

Several methods that include the Aux Pump Direct Inject feature have been included in the BioLogic DuoFlow Method Templates (see Section 6.2 and the online help for more information). This feature may be added to any protocol.

1. In the protocol load/inject step select Direct Inject as the injection type.
2. Select the Aux Load Pump option and set the sample volume, flow rate and flow direction. Note that if an EP-1 or non-Bio-Rad pump is used, the flow rate must be set both in this dialog and at the pump.
3. If an Aux Pump Inlet valve is connected, select the sample to load.
4. If the tubing is to be rinsed between sample injections add a second Load/Inject step, select the rinse buffer and set the rinse volume, flow direction and flow rate.

When doing Aux Pump Direct Injection the AVR7-3 injection valve operates as follows:

- **Load**: In this position, the valve connects ports 4 and 5 and is used for column equilibration and sample elution.
- **Inject**: In this position, the valve connects ports 3 and 4 and is used to inject sample onto the column.
- **Purge**: In this position, the valve connects ports 5 and 7 and is used to take the column out of line for purging or buffer changes.

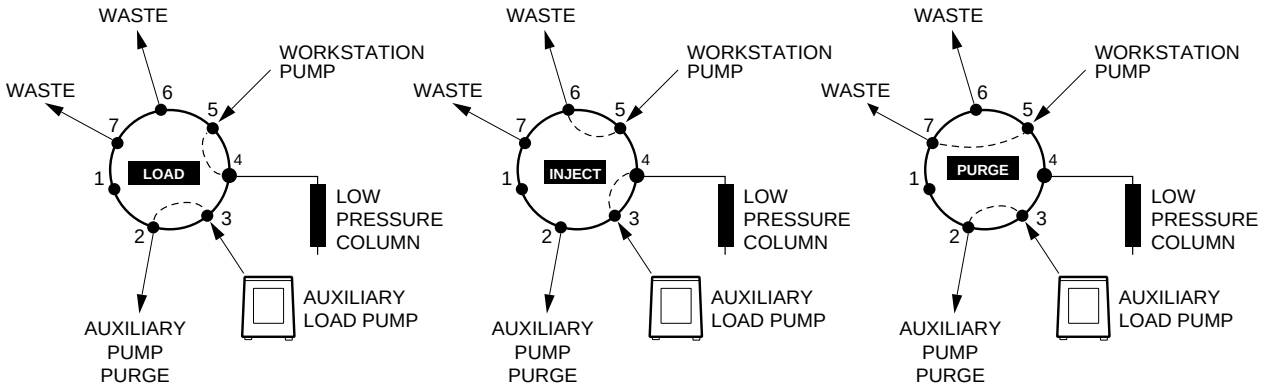


Figure 8-3. AVR7-3 Valve Positions During a Run with Direct Sample Loading and Injection

8.3 GRADIENT PUMP DIRECT INJECTION

Large volume samples can be directly loaded through the Workstation pumps. Up to 7 samples can be sequentially loaded if an inlet valve (AVR9-8) is added to the setup. One of the inlet valve ports must be assigned to the running buffer. Similarly, an SVT3-2 or SV5-4 Aux pump inlet valve may be used to load 1 sample or 3 samples, respectively. Samples loaded through the pumps should be filtered through a 0.45 um filter. Furthermore, the pump should be washed with a sanitization solution afterwards to remove residual protein contamination that could reduce the piston seal lifetime. Consult the DuoFlow online help for additional information.

System Setup

1. Connect a valve (SVT3-2, SV5-4 or AVR9-8) to Inlet A or B as shown in Figure 8-4. For systems configured with a Maximizer, valves can be connected at Inlets A1, A2, B1 and B2.
2. In the device setup screen, define a connected valve (SVT3-2, SV5-4 or AVR9-8) as an Inlet A (or Inlet B) valve and name each valve position. As shown in Figure 8-4, one position (usually position 1) should be defined as the running buffer. To automate system cleaning, ports may also be assigned to sanitization and storage solutions.
3. To prevent air from entering the system, unused valve ports should be plugged with the ¼-28 plugs supplied in the DuoFlow fittings kit.

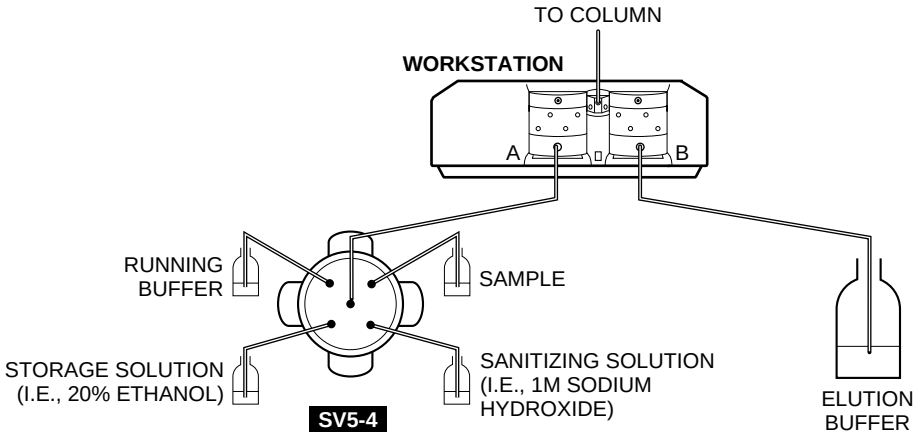


Figure 8-4. Sample Loading Through the Workstation Pump

Writing the Gradient Pump Direct Inject Protocol

The protocol Load/Inject step should be programmed as follows:

1. In the protocol Load/Inject step, select Direct Inject as the injection type, select the Pump A (or Pump B) option, select a sample and set the sample volume and flow rate.
2. Add an isocratic step after the load step to ensure that the entire sample is loaded onto the column. The inlet tubing and mixer should be rinsed with at least 5 volumes of running buffer to ensure that the entire sample is loaded onto the column.
3. Add protocol cleaning steps to ensure that all the residual protein has been removed from the tubing and mixer.
    a. Add a Change Valve step to move the AVR7-3 sample inject valve to the Purge position
    b. Add an isocratic flow step and run 3-5 volumes of Buffer B through pump B.
    c. Add an isocratic flow step and run 3-5 volumes of Buffer A through pump A
    d. Add a change Valve step to move the AVR7-3 sample inject valve back to the Load position.

System Sanitization

For sanitization, 1.0 M sodium hydroxide (NaOH) may be used. Be sure to wash with water to remove the base. Refer to Chapter 11, Maintenance, for information on how to store the DuoFlow system.

8.4 DYNALOOP SAMPLE INJECTION

The DynaLoop, which is available in 25 ml and 90 ml sizes, is used to load large volumes of sample directly onto chromatography columns. These loops have a sliding piston and function very much like a syringe. The sample is inserted into the DynaLoop's sample end connector. The loading of the sample pushes the DynaLoop's sliding seal assembly towards the buffer end connector. **Note:** While other dynamic loops may be used, the application discussed in this section applies specifically to the DynaLoop.

System Setup

When plumbed directly to the AVR7-3 inject valve, the DynaLoop functions just like a static sample loop. The sample end of the DynaLoop should be plumbed to port 3 and the buffer end to port 6 of the Inject valve. The valve's operation and sample loading are controlled automatically, simplifying the sample injection process and insuring precise sample loading and gradient formation.

The DynaLoop may be filled either manually with a syringe or automatically using an auxiliary pump such as an Econo Gradient Pump (EGP) or EP-1 Econo pump that is controlled by the DuoFlow system.

- When using a syringe to fill a DynaLoop, the syringe should be connected to port 2 of the AVR7-3 valve. However, using a 1/4x28 to female Luer adapter between the syringe and the valve makes filling the loop much easier.
- When using the auxiliary pump to fill the loop, the pump's outlet plumbing should be connected directly to port 2 of the inject valve.

Consult the DynaLoop Instruction Manual or DuoFlow online help for additional information. The figure below shows how to set up a DynaLoop.

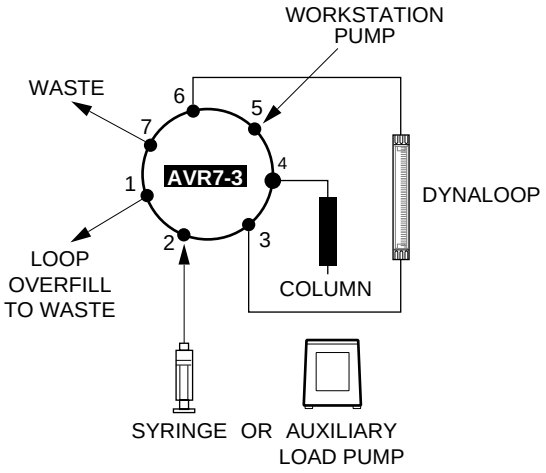


Figure 8-5. Plumbing the DynaLoop for use with an Inject Valve

Writing the Protocol

Before running a protocol which involves the use of a DynaLoop, be sure to purge air from the lines, as discussed in the DynaLoop Instruction Manual, Chapter 2, Installation. Then follow the guidelines which apply to your application.

The following steps should be programmed within the method protocol to manually load a sample:

1. Ensure that the column is properly equilibrated by using an **Isocratic Flow** step.

2. From the Manual screen, set the AVR7-3 sample inject valve to its Inject (I) position and allow the sliding seal to contact the sample end fitting on the DynaLoop using the purge procedure discussed in the Dynaloop Instruction Manual. Then set the valve to the Load (L) position.

3. Load sample into the DynaLoop by using a syringe. As sample is loaded into the loop, it displaces the buffer that was used to purge the unit. This design means the DynaLoop cannot be overfilled.

   When sufficient volume of sample for a run or series of runs is loaded into the DynaLoop, stop the filling process. For a series of partial volume injections after one loading sequence, fill the DynaLoop with about 2 percent extra sample. Leave the syringe in the Inject port to minimize the introduction of air into the DynaLoop.

   The sample is now ready for injection, and the protocol can be programmed.

4. In the Protocol screen, select **Load/Inject Sample**. In the Load/Inject Sample window, note that the AVR7-3 valve will automatically move to the “Inject” position at the start of the step and to the “Load” position at the end of the step.
   a. In the Load/Inject Sample window, specify Dynamic Loop.
   b. In the “Injection Buffer” area of the window, select the buffer(s) and percent (%) composition to be used to inject the sample onto the column.
   c. In the “Volume (ml)” field, enter the sample volume.
   d. In the “Flow (ml/min),” enter the flow rate of the Workstation pump that will be used to inject the sample onto the column.

5. Continue writing the separation protocol.

The following steps should be programmed within the method protocol to automatically inject a sample using an auxiliary pump:

1. Connect the Aux pump. The EGP pump connects to the Instrument bus. If you are using the EP-1 Econo pump, consult Section 8.2.

2. In the Setup screen, select Aux Load Pump and AVR7-3 valve in addition to the other device and instruments you have connected to the system.

3. In the Protocol screen, program an **Isocratic Flow** step that is long enough for the auxiliary pump to load the desired quantity of sample into the DynaLoop. For example, if you are loading 25 ml of sample at an auxiliary pump flow rate of 5 ml/min, you will need at least a 5 minute step prior to the sample loading step. Otherwise the protocol will fail the automatic validation done prior to the run. The flow rate of the DuoFlow pump is not critical, so you may set a low flow rate (e.g., 0.1 ml/min) to minimize buffer waste. It is important that either the time or volume length of this first step is of sufficient duration to allow the auxiliary pump to fill the DynaLoop.

4. In the Protocol screen, select **Load/Inject Sample** to program the DuoFlow system to automatically fill and inject the DynaLoop sample. From the Load/Inject Sample window,
   a. Select the Dynamic Loop as the type of loop to be used.

b. Select **Fill Before Inject**. This instructs the auxiliary pump to load the sample into the DynaLoop. Note that the auxiliary pump flow rate is not under DuoFlow control. The flow rate is used by the system when validating the protocol before the run. Because the DuoFlow system only starts/stops the auxiliary pump, the correct flow rate must be set at the auxiliary pump. The flow rate of the Econo pump is set from the pump and is recorded in the yellow data entry boxes of the Fill Sample section of the Load/Inject Sample dialog box. Note that the rinse function is not available when the DynaLoop is being used.

c. In the Fill Sample Loop area of the window, select the sample to be loaded and enter its volume and flow rate of the auxiliary pump.

d. In the Inject Sample area of the window, select the Injection Buffers, the buffer composition, the flow rate of the Workstation pumps, and the sample volume to be injected onto the column.

The DuoFlow system will now automatically control the loading and injection of the sample. Note that the rinse function is not available when the DynaLoop is being used.

5. Continue writing your desired separation protocol.

During the run, the valve functions as follows:

- Load. While in this position the valve connects ports 5 and 4 for equilibration of the column and for sample elution. In this position, sample loop is loaded to the desired volume via port 2 and buffer is expelled from the dynamic loop through port 1.
- Inject. While in this position the valve connects ports 5 and 6 and ports 3 and 4 for applying the sample onto the column. The flow from the Workstation pump forces the sliding piston to expel the sample onto the column.
- Purge. While in this position the valve connects ports 5 and 7 and allows purging or buffer changes of the Workstation pump without the need to remove the column from the system.

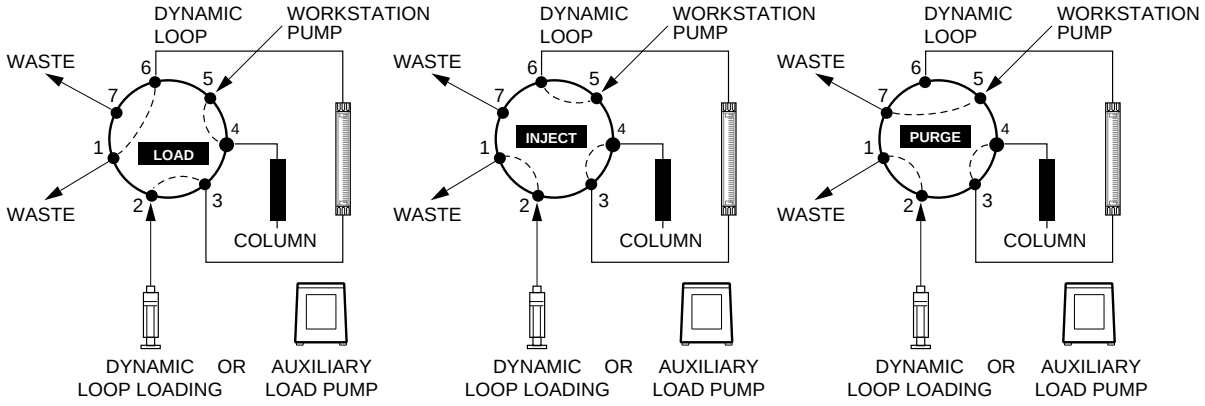


Figure 8-6. Valve Positions During a Run using the DynaLoop

9.0 COLUMN AND BUFFER FLOW SWITCHING APPLICATIONS

9.1 COLUMN SWITCHING

A Column Switching valve is used to connect multiple columns to the BioLogic DuoFlow system. Inclusion of a column-switching valve in the setup can be an advantage if you routinely use a variety of columns on your system. One application of this feature includes the running of multiple samples (see Section 8.1 and 8.2) where each sample requires its own column. Another application is the running of two and three- dimensional chromatography experiments where the sample eluted from one column is subsequently injected onto a second or third column. There are two types of column switching valves that can be used with the DuoFlow system: AVR7-3 two-column switching valve and an AVR9-8 eight-column switching valve (requires 2 AVR9-8 valves).

9.1.1 AVR7-3 Two-Column Switching

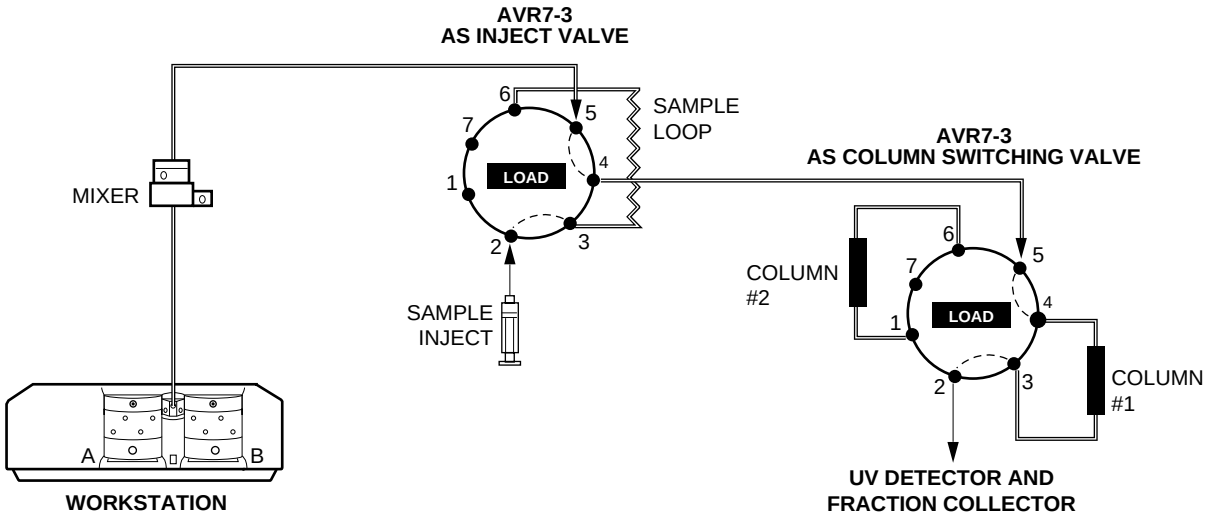


Figure 9-1. Column Switching Using Two AVR7-3 Valves

For this application, an AVR7-3 column-switching valve is required in addition to the AVR7-3 sample inject valve.

System Setup

1. Plumb an AVR7-3 column-switching valve as shown in Figure 9-1. Column 1 should have its inlet connected to port 4 and outlet to port 3. Column 2 should have its inlet connected to port 6 and outlet to port 1.
2. Plumb port 5 of the column-switching valve to port 4 of the upstream sample inject valve.
3. Plumb port 2 of the column-switching valve to your detector.
4. In the device setup, configure the AVR7-3 valve as a user-define valve and name the valve "Column Switcher". Name valve positions 1, 2 and 3 as Column 1, Column 2 and Purge, respectively.

Writing the Column Switching Protocol

When using multiple columns, care should be taken to prevent cross-contamination. Purge steps should be included before placing a column inline to ensure that the tubing is clean and filled with the appropriate buffer. The following three steps should be included as part of a column-switching step.

1. Add a Change Valve step to your protocol and place the Column Switcher valve in the Purge position.
2. Add an isocratic flow step to wash the tubing and mixer with 3-5 volumes of buffer.
3. Add a Change Valve step to your protocol and select the desired column on the Column Switcher valve.

Column-switching steps are usually placed at the beginning of a protocol or at the start of each dimension in a multi-dimensional chromatography experiment.

9.1.2 AVR9-8 Eight Column Switching

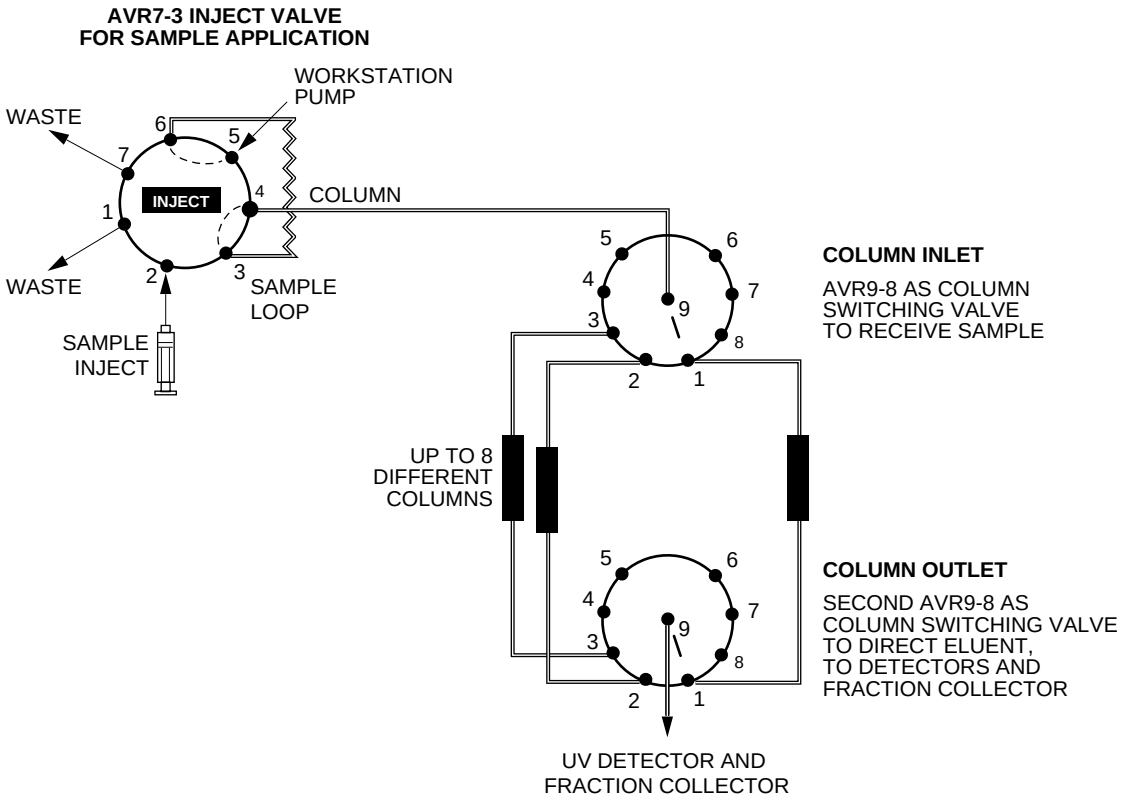


Figure 9-2. Column Switching using Two AVR9-8 Valves

For this application, two AVR9-8 valves are required.

System Setup

1. Plumb two AVR9-8 valves as shown in Figure 9-2. Each column inlet should be connected to the column inlet valve and each column outlet to the column outlet valve. Unless eight columns will be connected, a length of tubing should be plumbed between the two AVR9-8 valves at position 1 to be used as a column bypass. This facilitates washing of the system between column switching events. Column bypass tubing or ¼-28 plugs should be placed at each unused valve port.
2. Plumb the common port of the column-switching inlet valve to port 4 of the sample inject valve.
3. Plumb the common port of the column-switching outlet valve to your detector.
4. In the device setup, define an AVR9-8 valve as a Column-Switching valve. In the AVR9-8 valve dialog identify the ports where the two AVR9-8 valves are connected to the Workstation or Maximizer and name each valve positions, e.g. "Column Bypass", "Column 1, "Column 2" etc.

Writing the Column Switching Protocol

When using multiple columns, care should be taken to prevent cross-contamination. Purge steps should be included before placing a column inline to ensure that the tubing is clean and filled with the appropriate buffer. The following three steps should be included as part of a column-switching step.

1. Add a Column Switching step to your protocol and select the Column Bypass position.
2. Add an isocratic flow step to wash the tubing and mixer with 3-5 volumes of buffer.
3. Add a Column Switching step to your protocol and select the desired column.

Column-switching steps are usually placed at the beginning of a protocol or at the start of each dimension in a multi-dimensional chromatography experiment.

9.2 REVERSE FLOW CHROMATOGRAPHY

Reverse flow chromatography is generally used in affinity chromatography experiments to prevent peak broadening. In affinity chromatography most protein sticks to the top of the column and is eluted off through the column. This results in band broadened due to diffusion. In reverse flow chromatography, molecules are eluted directly off the top of the column and do not pass through the column. This results in sharper bands. For this application, an AVR7-3 reverse flow valve, in addition to the AVR7-3 sample inject valve, is required.

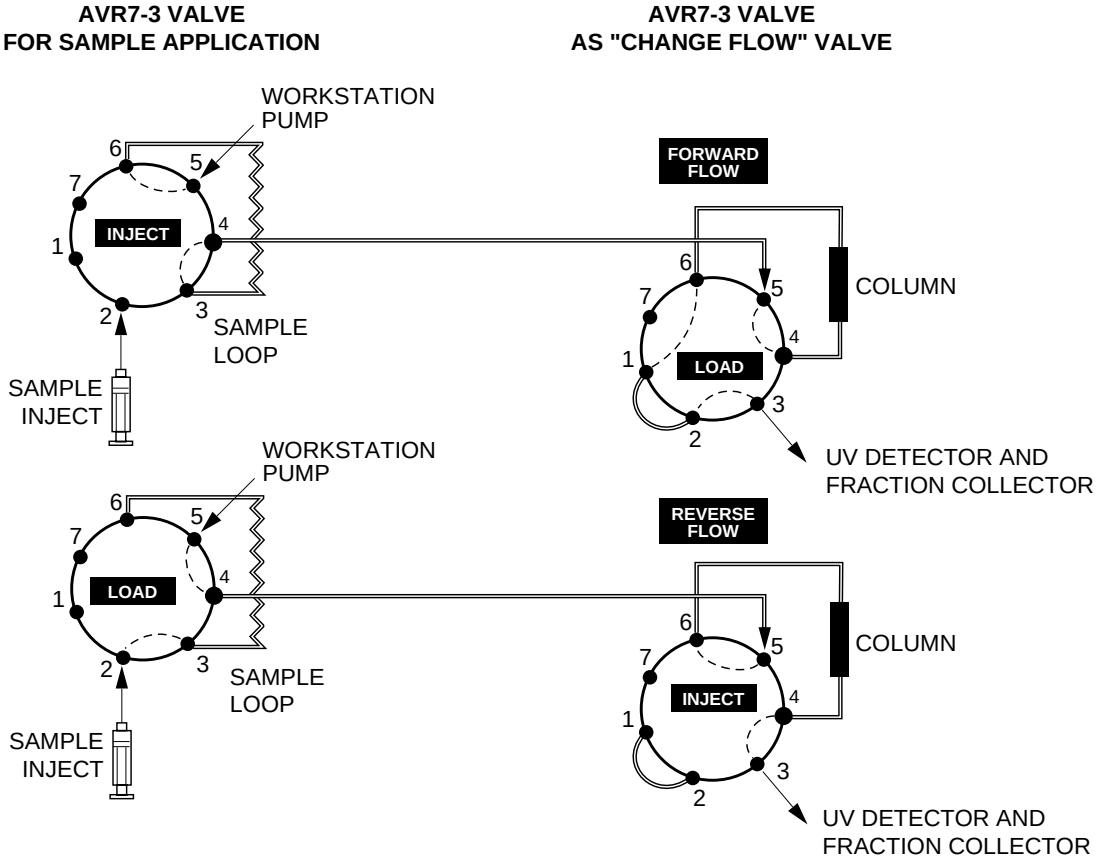


Figure 9-3. Reverse Flow Affinity Chromatography with an AVR7-3 Valve

System Setup

1. Plumb an AVR7-3 reverse flow valve as shown in Figure 9-3. The column should be connected between port 4 (column inlet) and port 6 (column outlet).
2. Plumb a short piece of tubing between ports 1 and 2 of the reverse flow valve.
3. Plumb port 5 of the reverse flow valve to port 4 of the upstream sample inject valve.
4. Plumb port 3 of the reverse flow valve to your detector.
5. In the device setup, configure the AVR7-3 valve as a user-define valve and name the valve "Change Flow Valve". Name valve positions 1, 2 and 3 as "Forward Flow", "Reverse Flow" and "Purge', respectively.

Writing a Flow Switching Protocol

The following three steps should be included as part of a reverse flow experiment.

1. Add a Change Valve step at the beginning of the protocol and place the Reverse Flow valve in the Forward Flow position.
2. Just before the elution step add a Change Valve step to your protocol and place the Reverse Flow valve in the Reverse Flow position.

9.3 MULTI-DIMENSIONAL CHROMATOGRAPHY

Multi-dimensional chromatography is used to perform multi-step chromatographic purifications in a single automated run. This technique is particularly useful for purifications done on a routine basis. Multi-dimensional experiments usually involve some type of affinity purification step followed by a high-resolution step such as ion exchange, hydroxyapatite or size exclusion chromatography. Table 9-1 shows a few common multi-dimensional chromatography experiments types.

Table 9-1. Common Multidimensional Chromatography Experiments

Experiment Type	**Dimension 1**	**Dimension 2**	**Dimension 3**
2D	Affinity		Desalt
2D	Affinity		Size Exclusion
3D	Affinity	Desalt	Ion Exchange

Most of the common protein purification techniques can be incorporated into multidimensional chromatography experiments. Generally, purification techniques that are sample independent provide the greatest benefit when included as part of a multidimensional experiment. Examples include: affinity chromatography of proteins tagged with His tags, glutathione-s-transferase and maltose binding protein; size exclusion chromatography and ion exchange chromatography.

Both two- and three-dimensional chromatography experiments can be performed using the BioLogic DuoFlow system. The type and complexity of experiments that you can run will depend on the hardware installed on your system. Maximizer and Pathfinder systems, with their increased valve capacity, can run the most complex experiments.

System Setup

When setting up a multi-dimensional chromatography protocol it is important to consider a few essential factors. First of all, sequential purification protocols must be compatible. In other words, the material collected in one dimension must be suitable for injection in the next dimension in terms of composition, pH, concentration etc. Another factor to consider is the number of fractions to be collected and the capacity of your fraction collector. Although fractions can be collected during the entire experiment it is usually best to only collect fractions during the final dimension in order to avoid running out of tubes. This is particularly important if you are purifying multiple samples.

The setup of a multidimensional experiment can be complex and requires that care be taken in plumbing the valves and equilibrating the system. The placement of valves, pumps and detectors should be planned so as to minimize tube lengths. Prior to connecting columns to the system make sure that all the tubing has been filled with buffer.

A few plumbing diagrams have been supplied in the BioLogic software online help that show how to plumb both two and three-dimensional chromatography experiments. Method templates designed for these plumbing diagrams are available in a file named "Multi-D Templates.zib" located in the BioLogic directory.

Writing the Protocol

The powerful queuing feature of the BioLogic software makes writing multi-dimensional chromatography experiments simple. Multi-dimensional experiment can be written as a series of individual one-dimensional chromatography methods that are then ran sequentially as part of a queued run. Once written, each one-dimensional method can be combined with other methods to make the desired experiment. For example, a single affinity protocol could be could be combined with either a desalting method or size exclusion method to create one of the two-dimensional experiment shown in Table 1. On the other hand an affinity method could be combined with both a desalting method and an ion exchange method to create the three-dimensional experiment shown in Table 1.

When writing a multi-dimensional method, care should be taken to prevent reintroduction of impurities removed in previous dimensions. Cleaning and equilibration steps should be included at the start of each dimension to wash any impurities from the tubing and to fill the system with the appropriate buffers.

10.0 BUFFER BLENDING

The Maximizer is a component of the DuoFlow system that dynamically "Blends" water, salt and the conjugate acid and base of a buffer to produce a solution with a specific salt concentration and pH. Buffer Blending is a powerful chromatographic tool that in conjunction with the scouting feature of the DuoFlow software can be used to optimize salt concentration, gradient slope and pH in a set of unattended experiment. The results of these experiments can then be easily compared and analyzed using the Trace Compare feature of the DuoFlow software.

Chromatography with a Maximizer offers three major benefits. First is its "Buffer Blending" feature that allows the composition of the pump effluent to be defined in terms of pH and salt concentration from a single set of reagents. Buffers at any pH within the pH range of the buffer (typically pKa ± 1) can be obtained. Second, solution preparation is simplified since buffer salts need to only be weighed out and diluted to the appropriate concentration. There is no need to adjust the pH of the solutions. The third benefit of using a Maximizer is that it doubles the accessible flow rates. This occurs since gradients are no longer made by the pumps but by the Maximizer proportioning valves. This allows both pumps to run at their full speed.

10.1 DOUBLED FLOW RATE CAPACITY USING A MAXIMIZER

The Maximizer valve system doubles the DuoFlow system flow rate capacity from 10 ml/min (F10 pump head) and 40 ml/min (F40 pump head) to 20 ml/min and 80 ml/min respectively. This is accomplished since the Maximizer proportioning valves, rather than the pumps, are used to change buffer composition (percent Buffer A and Buffer B). This doubled flow rate capacity is available only when Buffer Blending is defined in the Device Setup. The Buffer Blender feature can be used in two different ways. 1) "Buffer Blending", where water, salt and the conjugate acid and base of a buffer are combined to produce a solution with a specific salt concentration and pH, or 2) "Non-Blending", where two solutions of fixed composition (i.e. Buffer A = 25 mM Tris pH 8.1 and Buffer B = 25 mM Tris pH 8.1 + 1 M NaCl) are mixed to produce a solution with a specific salt concentration.

Buffer Blending. In "Buffer Blending", proportioning valve A is used to control the amount of acid and base added and valve B is used to control the amount of water and salt added. The effluent from pump A and B are then combined in the mixer. In "Buffer Blending", the effluent from pumps A and B dilute each other and so each solution must be prepared at a 2X concentration. Buffer Blending allows buffers of any pH within the buffer pH range to be produced. Buffer Blending is discussed further in Section 10.2.

Non-Blending. In "Non-Blending", both proportioning valves have the same function and are each used to control the percent Buffer A and Buffer B in the pump effluent. In this case, the effluent from pump A and pump B are identical so no dilution occurs when they are mixed. Therefore, the buffers can be prepared at their 1X concentration. The Buffer Blender setup dialog includes a "High Flow Non-Blending (1x)" buffer system for use in situations where a high flow rate is required but where "Buffer Blending" is not needed. When using this method, inlet tubes A1 and B1 are placed in Buffer A and inlet tubes A2 and B2 are placed in Buffer B.

A second high flow non-blending buffers system titled "High Flow Non-Blending", has also been included in Buffer Blender buffer system list. This high flow non-blending method uses inlet valve A to load Buffer A and inlet valve B to control the percent Buffer A and B in the effluent. In this case, Buffer A is plumbed to inlets A1 and B1. Buffer B is plumbed to inlet B2 and must be prepare at 1X in buffer and 2X in salt. Inlet A2 is not used and should be plugged.

10.2 BUFFER BLENDING WITH THE MAXIMIZER

The BioLogic DuoFlow software provides 26 predefined buffer systems for use in Buffer Blending experiments (see Table 10-1). These buffer systems cover a wide pH range and include most of the commonly used chromatography buffers. Each buffer system includes recipe text that describes how to prepare the required Buffer Blending solutions. In addition, the Buffer Editor can be used to create user-defined buffer systems. The Buffer Editor is a simple tool used to edit (or modify) predefined buffer systems or to create new buffer systems. Buffer Blending solutions should always be made from reagent grade materials and then filtered and degassed before use. Filtering the solutions will increase the life of the DuoFlow check valves as well as the column. Degassing solutions will help prevent air bubbles from forming in the DuoFlow pumps and the detector.

Table 10-1
Buffer Blending Buffer Systems

	Buffer System	pH Range*
1	Acetate (20 mM)	3.7 to 5.7
2	Acetate (50 mM)	3.6 to 5.6
3	Bis-Tris (20 mM)	5.6 to 7.6
4	Bis-Tris (50 mM)	5.5 to 7.5
5	Citrate (20 mM)	2.6 to 6.0
6	Citrate (50 mM)	2.4 to 6.0
7	Formate (20 mM)	3.0 to 4.6
8	Formate (50 mM)	2.6 to 4.6
9	HEPES (20 mM)	6.4 to 8.4
10	HEPES (50 mM)	6.4 to 8.4
11	MES (20 mM)	5.1 to 7.1
12	MES (50 mM)	5.1 to 7.1
13	N-Methylpiperazine (pH 4.2 to 5.5)	4.2 to 5.5
14	Phosphate (20 mM)	5.8 to 7.5
15	Phosphate (50 mM)	5.6 to 7.5
16	Phosphate (100 mM)	5.5 to 7.5
17	Phosphate with Ammonium Sulphate	5.6 to 7.2
18	TAPS (20 mM)	7.4 to 9.4
19	Triethanolamine (20 mM)	6.8 to 8.8
20	Triethanolamine (50 mM)	6.8 to 8.8
21	Tris (25 mM)	7.1 to 9.1
22	Tris (50 mM)	7.1 to 9.1
	Broad Range Buffers*	
23	Bis-Tris/Tris (4 ºC)	6.2 to 9.4 (4ºC)
24	Bis-Tris/Tris (25 ºC)	5.8 to 8.9
25	N-methylpiperazine/Bis-Tris/Tris (25 ºC)	4.7 to 9.4
26	Formate/Acetate/Phosphate (25 ºC)	3.1 to 6.8

* pH range is at 25 C unless otherwise noted

The Buffer Editor is used to create new buffer systems or to edit existing buffer systems. Note that Read Only buffer systems cannot be modified. To modify a Read Only buffer system, uncheck the Read Only box or Edit a Copy of the buffer system. The following procedure should be used to create or edit a buffer system.

1. Define a buffer salt
    a. From the Buffer Editor main screen press Edit Buffer List
    b. Use the Buffer Salt Name list to select and view the desired buffer. If the buffer is in the list, and it is made from reagents available in your laboratory press Done and go back to the main screen.
    c. If the buffer is in the list but is made of reagents that are not available in your laboratory, press Copy, rename the buffer and change the buffer information as necessary. Save the buffer, press Done and go back to the main screen.
    d. If the desired buffer is not in the list, press New and enter the required buffer information. Save the buffer, press Done and go back to the main screen. The "Use Activity Correction" option is used to correct for the pKa dependence on buffer ionic strength. In practice, this option should be checked for all pKa's where the charge of one of the associated buffer ions is greater than 0 or less then –1.
2. Define a Salt
    a. From the Buffer Editor main screen press Edit Salt List.
    b. Use the Salt Name pull-down list to select and view the desired salt. If the salt is in the list and is made from reagents available in your laboratory press Done and then back to the main screen.
    c. If the desired salt is not in the list Copy an existing Salt or Press New to create a new salt. Enter the required information, save the salt and press Done.
3. Create or Edit a Buffer System
    a. From the Buffer Editor main screen press Create New Buffer System to create a new buffer system or press Edit Current Buffer System to copy and edit an existing "Read Only" buffer system.
    b. From the Buffer selection screen select the number of buffers that will be included in the buffer system (1, 2 or 3) and then select the buffer(s) and specify their 2X concentration. Press Next and to to the next step.
    c. Select a salt from the salt selection screen and specify its 2X concentration. Press Next and go to the next step.
    d. Select the batch size for the solutions that will be prepared. Note that this will automatically name each solution and generate the recipe text. Change the solution names and recipe text as desired and press Next and to go the next step.
    e. From the Save Buffer System screen, enter the required information and then press Save. The following information will be helpful in setting the pH range and reference temperature.
        - pH Range: The "View Table" feature can be used to estimate the buffer pH range. The usable pH range is generally between 10 % and 90 % A2 but also depends on the ionic strength (%B) of the buffer. The table will include user-defined pH corrections if you are editing an existing buffer system that is also currently defined in the method setup and uses pH corrections. User-defined pH corrections are stored in the method setup and not in the Buffer Editor.
        - Reference Temperature. This is the temperature at which the Buffer Blending tables are calculated (usually set to 25ºC except as noted below). The Maximizer uses the buffer temperature coefficient to correct the Buffer Blending tables from the reference temperature to the run temperature. In the case of multiple component buffers (buffers with more that one type of buffer), an average temperature coefficient is used to correct the tables. In this case the Reference Temperature should be set near the run temperature to obtain the best pH accuracy.

10.3 PH MEASUREMENT AND CORRECTIONS

Buffer pH plays an important role in chromatography of biomolecules since it affects both their stability and their chromatographic properties. Both solution temperature and ionic strength affect the pH of a buffer. The Maximizer is designed to compensate for both of these effects during an experiment. A sensor in the conductivity flow cell monitors temperature and so must always be connected to the Maximizer. Changes in pH due to changes in ionic strength are compensated for by information stored with each Buffer System. Changes in pH due to sample injection and/or adsorption or desorption of ions from a column cannot be compensated for and may cause the pH to deviate from the specified run pH. For this reason it is advisable to run ion exchange experiments at a pH near the middle of the buffers pH range where the buffer capacity is greatest.

Accurate pH measurements require that samples be of uniform composition and temperature. These conditions do not generally occur when monitoring pH during a chromatographic run. For this reason pH's measured during a run should always be considered approximate. Furthermore, some pH accuracy is sacrificed in order to make pH probes that are able to operate at the flow rates and pressures used in chromatography applications. If highly accurate pH measurements are required, collect fractions and measure their pH using a high quality pH probe (such as an Orion Ross electrode or other Tris compatible electrode) connected to a desk top pH meter.

The pH electrode supplied with the Maximizer is designed to give optimum performance during the inline measurement of pH. It can be used at flow rates up to 80 ml/min and pressures up to 75 psi. Its use provides useful information about the Maximizer performance and buffer composition during a run. However, it is **not required** for accurate pH delivery by the Maximizer. Since the pH probe has an approximately 80 ul flow cell volume it should be taken out of line when collecting small fractions sizes (i.e. when collecting in microplates).

Prior to use, the pH electrode should be calibrated using two standard solutions that span the pH range over which the experiment will be run (for example pH 7 & 4, pH 7 & 10 or pH 4 and 10). Calibration is performed from the Utilities/pH Probe Calibration menu option.

Although the Maximizer produces buffer with good pH accuracy, the accuracy can be improved by applying a 1 or 2-point correction using the DuoFlow software as described below.

Single point correction (best for isocratic experiments)

1. From the manual screen press the Buffer Blender "Setup" button and select the desired buffer system from the Buffer System list. Make sure the pH correction box is not checked. Press OK.
2. Set the desired pH, salt composition and flow rate and then press Start.
3. After the system has equilibrated collect some effluent.
4. Measure the pH using a high quality pH probe connected to a desk top pH meter that has been calibrated at the run temperature.
5. Load your experimental method from the Browser screen and press Setup.
6. Double click on the Buffer Blending icon, and select the desired buffer. The appearance of the Buffer Blending setup dialog depends on whether a single component (see Figure 10-1) or multiple component buffer is selected (see Figure 10-2).
7. Place a check in the pH correction box and make sure the two-point correction option is not selected.
8. Enter the pH that was measured in Step 4 in the Observed pH box and the desired pH in the Desired pH box and press OK. For multiple component buffers, enter the measured pH for each buffer component, in both the 0 %B and user-set %B pH correction boxes. The software will calculate the correction.

(Optional) Check the pH Correction

9. From the manual screen press the Buffer Blending "Setup" button and select the desired buffer system from the Buffer System list. Turn pH correction on and enter the Desired and Observed pH from step 8 above.
10. Set the pH, Salt composition and flow rate. Press run.
11. After the system has equilibrated collect some effluent and measure the pH.

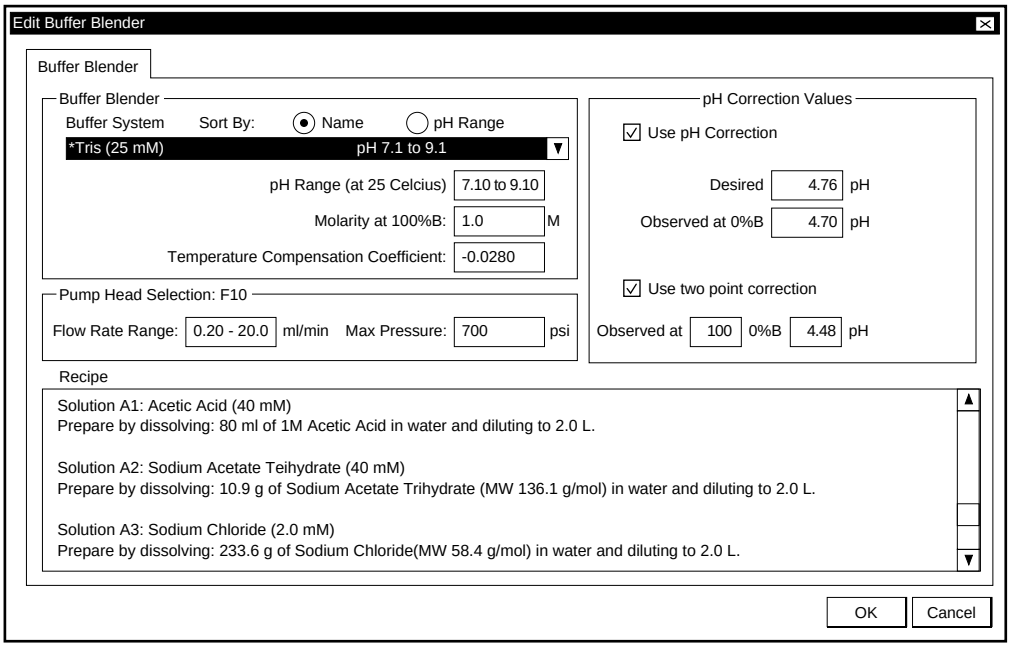


Figure 10-1. Buffer Blending Setup Dialog for a Single Component Buffer

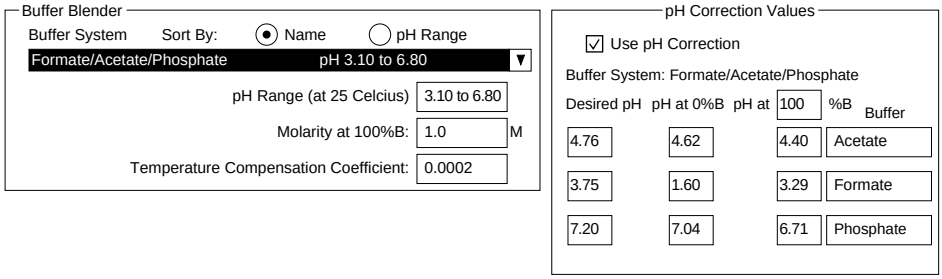


Figure 10-2. Buffer Blender Setup Dialog for a Multi-Component Buffer

Two point correction (best for gradient experiments)

1. From the manual screen Press the Buffer Blender "Setup" button and select the desired buffer system. Make sure the pH correction box is not checked.
2. Set the pH and flow rate. Set the salt composition to 0 %B. Press run.
3. After the system has equilibrated collect some effluent.
4. Set the salt composition to the maximum %B that will be used in the experiment. Press run.
5. After the system has equilibrated collect some effluent in a second tube.

6. Measure the pH of the low salt and then the high salt solutions using a high quality pH probe. Be sure to correct the reading for any difference between the sample temperature and run temperature. Note that if you are using a multi-component buffer system and want to use a buffer specific pH correction you will need to repeat steps 2 through 6 for each buffer component.
7. Load your experimental method from the Browser dialog and press Setup.
8. Double click on the Buffer Blending icon, and select the desired buffer. The appearance of the Buffer Blending setup dialog depends on whether a single component (see Figure 10-1) or multiple component buffer is selected (see Figure 10-2).
9. Place a check in the pH correction box and select the Two-Point Correction option.
10. Enter the desired pH in the Desired pH box.
11. Enter the low salt and high salt pH's that were measured in Step 6 in the Observed pH box and then enter the %B that was used to collect the high salt pH. For multi-component buffers repeat this for each buffer in the buffer system. The software will calculate the correction.

(Optional) Check the pH Correction

12. From the manual screen press the Buffer Blender "Setup" button and select the desired Buffer System from the recipe list. Turn pH correction on and enter the Desired and Observed pH from step 10 above.
13. Set the pH, Salt composition and flow rate. Press run.
14. After the system has equilibrated collect some effluent and measure the pH using a high quality pH probe.

11.0 MAINTENANCE

The BioLogic DuoFlow system requires minimal maintenance to assure reliable operation.

Note: Maintenance of the QuadTec UV/Vis detector and the Econo Gradient Pump (EGP) is discussed in their separate documentation.

11.1 CARE OF THE OUTER SURFACES OF THE INSTRUMENTS

During normal operation, spills and splashes may cause residues to form on the component cases.

To clean the case of an instrument, first unplug the instrument. Use a damp cloth to wipe down the outer case. Avoid wetting the power switch located below the front panel and the connectors on the rear of the unit.

The system rack tray should be mopped up and any residues rinsed away with water via the drain.

11.2 STORAGE OF THE DUOFLOW SYSTEM

For **overnight** storage, please note the following:

1. The system tolerates low salt buffer overnight, but sample and high salt buffers need to be washed out of the tubing.

2. Use deionizied (DI) water and the 10mL syringe provided in the Fittings kit to wash behind each pumphead with at least 10 ml of water. This applies to both the F10 and F40 pumps.

3. If the system is in a cold environment, keep it powered on to prevent condensation. If it is at room temperature, it may be left off or on. (When it is at room temperature, the DuoFlow Controller (computer and monitor) can be turned off, to conserve energy and prolong monitor life.)

4. Thoroughly flush all valves with water, especially the Maximizer valves and the SVT3-2 and SV5-4 solenoid valves.

For **long-term** storage, please observe the following precautions:

1. Follow the column manufacturer's instructions to clean and store the column. Remove the column from inline.

2. Run deionizied (DI) water through the entire system. Be sure to wash all wetted parts of both the Maximizer valves and the solenoid and automated valves to remove any contaminants.

   Note: It is particularly important to wash out the Maximizer valves and the SVT3-2 and SV5-4 valves. Failure to do so may result in valve failure.

3. Use deionizied (DI) water and the 10 ml syringe provided in the Fittings kit to wash behind each pumphead with at least 10 ml of water. This applies to both the F10 and F40 pumps.

4. Fill the entire system with either 20% ethanol (EtOH) or 0.05% sodium azide to inhibit bacterial growth. If necessary, fit the pump inlets, UV detectors and conductivity monitors, and all solenoid valves with 1/4-28 plugs which are provided in the Fittings kit.

11.3 CARE AND MAINTENANCE OF THE WORKSTATION PUMPS

The following three sections describe the procedures for system priming and periodic maintenance of the Workstation pumps.

11.3.1 Priming the Workstation Pumps and Removing Trapped Air Bubbles

> **Warning**: Do not run the Workstation pumps dry (without buffers in line), as this may result in damage to the pumpheads.

Care should be taken when setting up the DuoFlow system to ensure air does not get trapped in the pumps. Air trapped in the pumps can lead to erratic flow rates and poor gradient performance. To reduce the likelihood of bubbles getting into the pumps all buffers should be thoroughly degassed.

1. Ensure that all inlet tube fittings are securely fastened to the Workstation, Maximizer and valve inlets. Use the fittings tightener, supplied with the fittings kit, to tighten the fittings, if needed.
2. Immerse the Workstation pump A and B or Maximizer A1, A2, B1 and B2 inlet lines into filtered, degassed buffer.
3. Remove air from the inlet buffer lines and the pumps.
    a. Place the 10 ml luer syringe (supplied with the fittings kit) in the priming port of pumphead A. If a Maximizer is connected, select Inlet A1.
    b. Turn the priming port counter-clockwise to open the port and gently draw buffer into the syringe from the pumphead.
    c. Repeat this operation until no air bubbles are visible in the inlet tubing.
4. Remove air trapped behind the pump heads. This procedure should also be done as part of the instruments daily maintenance and anytime there are pressure fluctuations greater than ± 10%.
    a. Disconnect the pumphead outlet tube and hold an empty beaker up to the outlet port.
    b. With the syringe connected as described in step 3, inject buffer into the priming port using several short pulses to dislodge any trapped air and to push the air out of the pump head.
    c. Once all the bubbles have been dislodge, close the priming port and reconnect the pumphead outlet tube.
5. Repeat this priming procedure for the pump B inlet or inlets A2, B1 and B2, if a Maximizer is connected.

Pumping 200 ml of 100% methanol (MeOH) through each pumphead at 5 ml/min can greatly reduce air bubbles. Immerse inlet lines from both pumps into the methanol and pump at 50% B with the 40 psi backpressure regulator in place.

11.3.2 Daily Maintenance

The pumpheads should be washed daily with water when high salt buffers are used. This can be automated through the use of an SVT3-2 valve connected between the high salt buffer (buffer B) and pumphead B. Use one of the valve inlet ports to run the buffer and use the other inlet port to run water through the pumphead. If the system is configured with a Maximizer (non-blending mode) use the B1 and B2 inlet valves to run water (B1) or buffer (B2) through the pumphead.

Washing behind the piston seal extends seal life. Insert a 10 ml syringe filled with deionized water in the hole at the top of the pumphead. A 10 ml syringe is provided in the Fittings kit. Each pumphead should get a 10 ml washout at the end of the day's operation. Run off water exits through the washout drain between the two pumpheads. It can be collected in a small beaker.

11.3.3 Routine Maintenance of the Workstation Pumps

The Workstation pumps require minimal maintenance to stay in good working condition. The DuoFlow F10 Pump and F40 Pump Maintenance kits include the piston seals, check valves, and O-rings for routine maintenance of the pumps. Indications that maintenance is required include flow rates that are below the specified rate, an erratic delivery of liquid, liquid running out of the washout drain during normal operation (often seen as salt crystal accumulation), and poor gradient performance.

Safety: Make sure that any hazardous material has been flushed from the system, the pumps are not running, and any residual pressure has been bled from the system. Disconnect the Workstation power cord. Wear eye protection, gloves, and other protection as appropriate.

1. **Replacing a piston seal.** (Refer to Figures 11-1 and 11-2.)

   Each pump consists of PEEK pump blocks and screws, piston seals, O-rings and check valves.

   This procedure will require new piston seals. See Appendix D, Ordering Information.

   a. Soak the piston seals in 50% methanol to thoroughly wet them; sonicate in 50% methanol, if possible.
   b. Disconnect all tubing from the pumphead.
   c. Using the pumphead disassembly Allen wrench (or any standard 9/64” Allen wrench), remove the four long screws on the face of the pumphead center block (see Figure 11-1). Pull the pumphead away from the Workstation.
   d. Note that there are two piston assemblies per pumphead (see Figure 11-2). Each piston assembly consists of a piston, piston guide, and spring. Use a lab tissue to avoid getting fingerprints or oils onto the piston assembly. Carefully remove each piston assembly from its piston well in the pumphead assembly and set it aside.

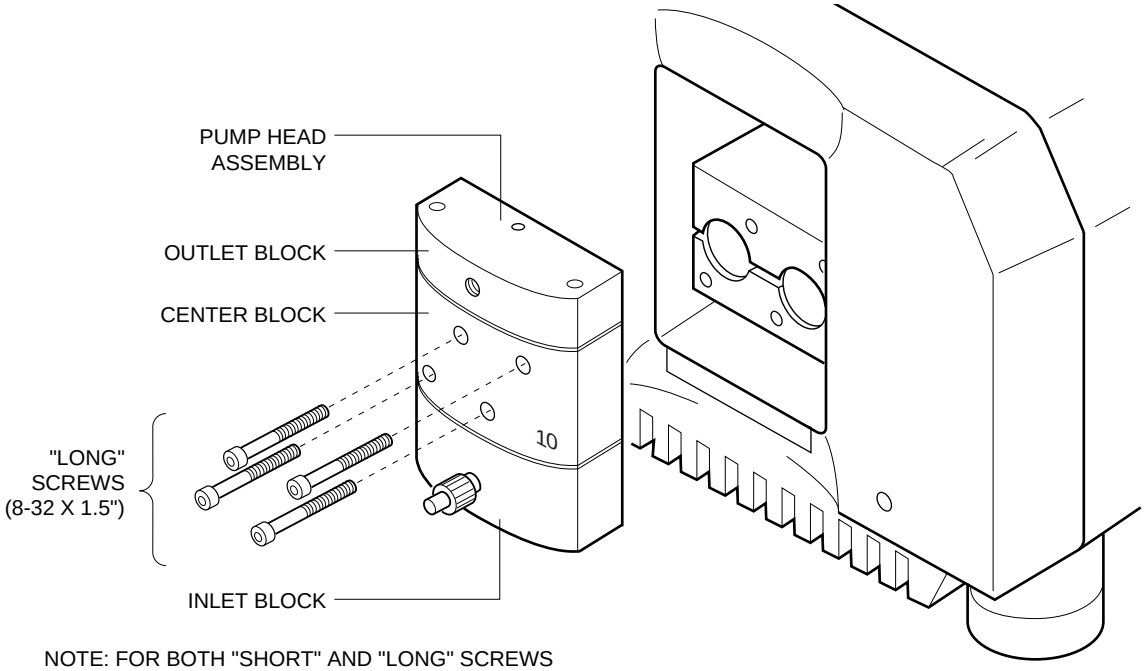


Figure 11-1. Workstation Pump Mechanism Parts

   e. Note the piston seal in each piston well. Always replace both seals at the same time.

      Using the piston seal removal tool, pry the seal from the piston well. To do this, you insert the tip of the seal removal tool into the seal and gently pry the seal away. Once a piston seal is removed, it cannot be reused.

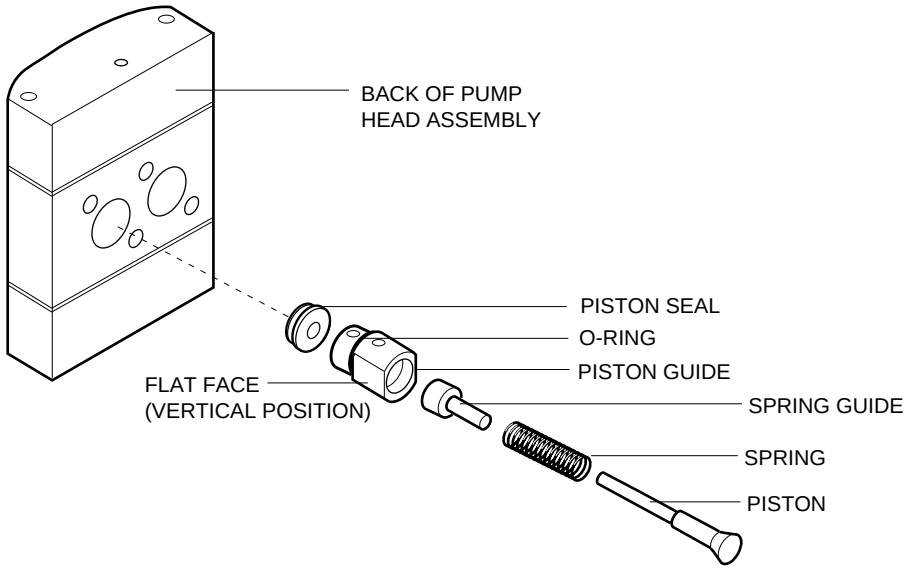


Figure 11-2. Piston Assembly and Access to the Piston Seal (view showing the back of the Pumphead Module)

f. Using your finger, press each new piston seal into its recess. Use the piston guide to press the seal completely into its recess.

g. Wipe the pistons with a dampened cloth before returning them to the piston wells. This ensures that no crystallized salt residues are present which could damage the new seals.

   When returning each piston assembly to its piston well, make sure that the seals in the piston wells on the pumphead assembly are securely mounted in place. The piston assemblies should be oriented so that the flat faces on the piston guides are in the vertical position.

h. Using the four long mounting screws removed earlier, remount the pumphead module to the Workstation so that it is secure. Insert and tighten the mounting screws. ***Do not over tighten.***

i. Condition the seal by pumping 100 ml of 100% methanol through each pumphead with the 40 psi backpressure regulator in place.

l. Flush the system with water, and then fill and flush with the buffers to be used for the separation.

2. Cleaning and replacing a check valve.

a. Soak the new check valves in 50% methanol to wet them. Sonicate in 50% methanol, if possible.

b. Remove the two short screws at the top of the pumphead assembly and the two long screws at the bottom of the pumphead assembly. Each pump separates into three blocks, revealing four check valves and two O-rings.

c. Replace the check valves. It is not necessary to replace the O-rings, as they are not subjected to high pressure.

d. Reassemble the pumphead. (Refer to Figure 11-3.) Check valves fit into the pumphead module in one direction only.

e. Wipe the pistons with lab tissue before returning them to the piston wells, and rinse the piston guides in running water.

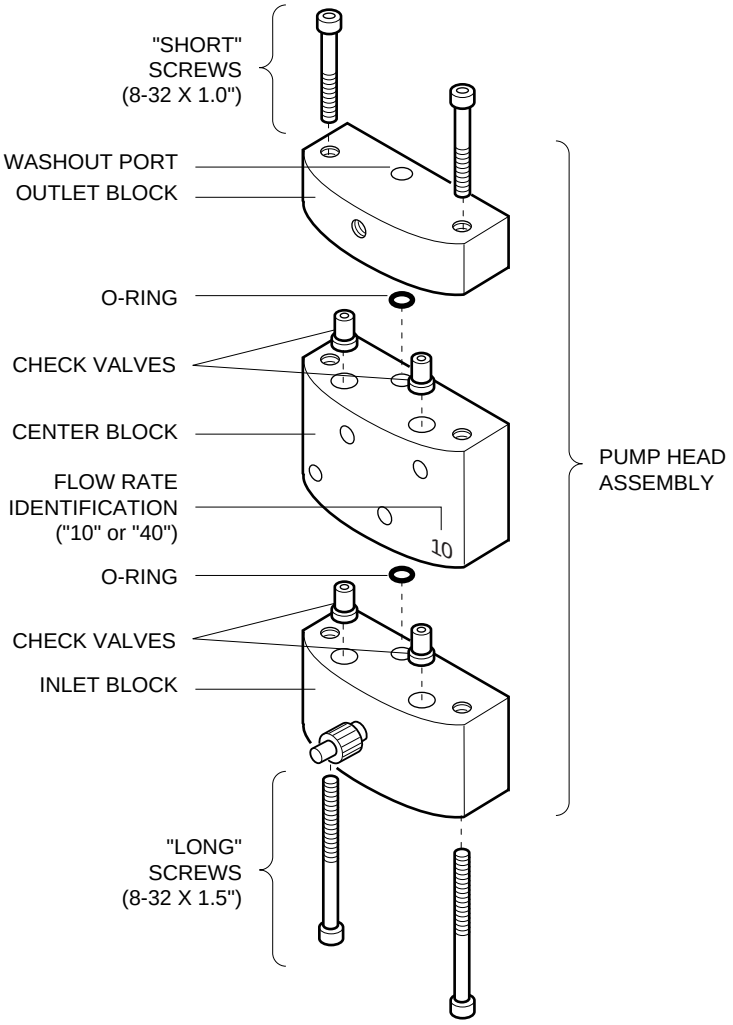
   The piston assemblies should be oriented so that the flat faces on the piston guides are in the vertical position.

f. Using the four long mounting screws removed earlier, remount the pumphead assembly to the Workstation so that it is secure. Insert and alternately tighten the mounting screws. Do not over tighten.

g. Using the priming port on the front of the pumphead, purge the pump with 50% methanol and then flush with water.

3. Care of the Pistons.

To maintain pump performance, the pumphead should be rinsed on a nightly basis. The pump may be rinsed automatically by programming a water rinse step at the end of each protocol. It is also important to rinse behind the piston seals by injecting water through the washout port, located at the top of the pumphead. A standard syringe may be used for this purpose.



NOTE: FOR BOTH "SHORT" AND "LONG" SCREWS USE PUMPHEAD DISASSEMBLY ALLEN WRENCH (OR STANDARD 9/64" ALLEN WRENCH)

Figure 11-3. Pumphead Assembly

11.4 MAINTENANCE OF THE UV DETECTOR AND THE CONDUCTIVITY FLOW CELL

The following two sections discuss the cleaning of the UV detector and the Conductivity flow cell and replacement of the lamp in the UV detector.

11.4.1 Cleaning the UV Detector and the Conductivity Flow Cell

Cleaning of the UV flow cell is indicated when the baseline is very noisy or unstable yet air bubbles have been removed from the flow cell. However, problems with UV detection during medium pressure chromatography are often solved by a few simple precautions. Air bubbles trapped in the flow cells give rise to a “sawtooth” signal pattern that will never stabilize. We recommend always running with a backpressure regulator after the detector (if using low pressure columns, place the backpressure regulator between the pump outlet and mixer). Equilibrate the column for at least 30 minutes to allow all bubbles to clear. Always degass buffers before use.

Make sure all fittings are tight to prevent leaks. If liquid leaks down between the flow cell and the optics module case, the cell should be removed and dried with a stream of pure nitrogen (never use compressed air). The case should be dried using a warm air blower or left for several hours to completely dry.

Air bubbles trapped in the Workstation pump give rise to erratic flow rates that are sometimes interpreted as problems with the UV detector. Refer to the previous section.

If cleaning of the UV detector and the Conductivity flow cell is desired, note the following:

- Filters: The standard filter tray contains both a 280 nm and a 254 nm filter. To clean the filters, loosen the filter’s thumbscrew and lift out the filter holder. The filters should be cleaned only when necessary using a dry lens tissue. To remove greasy fingerprints, use dilute ethanol or isopropanol solutions.
- UV Flow Cell: To remove the flow cell, undo the flat bottom fittings at the top and bottom of the flow cell. Then loosen the thumbscrews and push out the flow cell from the bottom. The flow cell is keyed to mount in one way only, so that it cannot be incorrectly mounted. Avoid touching the quartz windows with fingers.

  If the flow cell is visually dirty, it should be cleaned with one or more of the following solvents: dilute sodium dodecyl sulfate (SDS), 1M hydrochloric acid (HCl), 1M sodium hydroxide (NaOH), ethanol, or acetone. Run the solution through the flow cell using a syringe and leave for no more than 5 minutes. Rinse extensively with water and then blow dry using a gentle stream of pure nitrogen. **Never** dry with compressed air from a “house” line as this will contain microdroplets of oil that will coat the cell.

  When the detector is not in use, disconnect the flow cell and use a syringe filled with distilled water to clean out traces of salts and protein.

  Before storing the flow cell, inject a dilute solution (10% to 25%) of ethanol or isopropanol into the cell to prevent microbial growth. Use the plugs provided with the flow cell to seal the flow cell’s inlet and outlet lines.
- The Conductivity flow cell requires little maintenance other than rinsing routinely with deionized water.

11.4.2 Replacing the Lamp in the UV Detector

The following information covers the replacement procedures both for a new mercury lamp unit and a new zinc lamp unit. Note that the zinc lamp assembly has cooling fins and is larger than the mercury lamp assembly.

Indications that a lamp replacement is necessary include an unstable baseline and a decreased response to a standard concentration of a test chromophore. In this latter case, it is advisable to ensure that the flow cell is clean and that the correct wavelength filter is chosen before replacing the lamp. Refer to the previous section.

Note: As indicated during the following procedure, make sure the DuoFlow Workstation is turned off. This will eliminate the risk of electric shock, thermal burns, or UV light exposure to you or damage to the detector during cable connection.

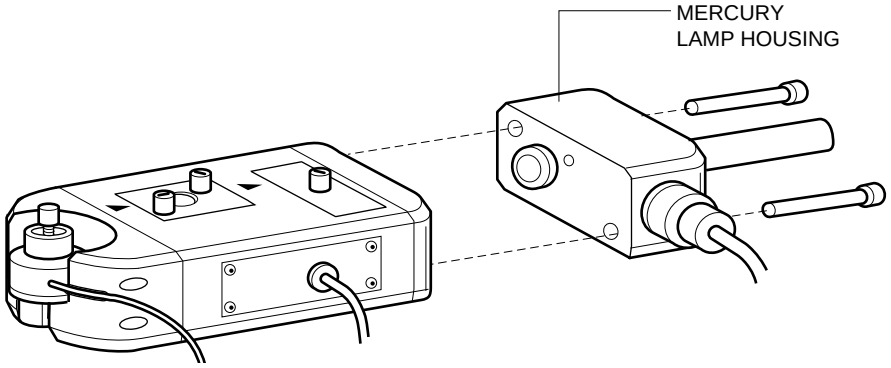


Figure 11-4. Replacing a Mercury Lamp in a Model OM-II UV Detector

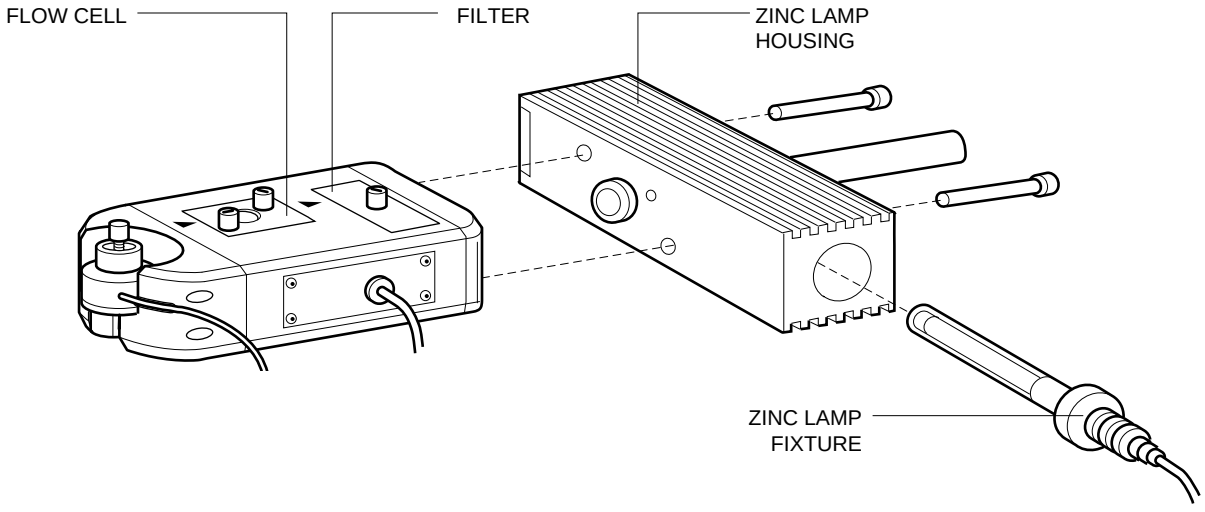


Figure 11-5. Replacing a Zinc Lamp in a Model OM-II UV Detector

1. At the DuoFlow Controller go to the Manual screen and turn off the lamp. Turn off power to the Workstation and unplug the UV Lamp cable, which is plugged into the UV Lamp connector on the rear of the Workstation.

2. Without disconnecting the lamp housing from the UV bench, undo the two small Allen screws securing the lamp fixture and gently pull the lamp out of the housing.

3. Remove the lamp unit from its packing material.
   WARNING: Do not touch the glass part of the lamp. Oils from you fingers will degrade the lamp over a period of time.

4. Carefully insert the replacement lamp fixture. The replacement lamp can be installed only in one orientation due to a small guide which fits into the housing. Secure it using the Allen screws for that purpose. Note that extra screws are provided with the kit.

   Note: Check that the O-ring seal at the lamp housing aperture is present and undamaged. If there is any indication of damage, or if it is missing, be sure to replace it using the O-ring supplied with the kit.

5. Reconnect the UV Lamp cable to the Workstation.

6. Turn on electrical power to the Workstation and at the DuoFlow Controller go to the Manual screen and turn on the lamp. Allow at least 30 minutes for the mercury lamp to warm up and at least several hours for the zinc lamp.

11.5 MIXERS

The Maximizer mixer and the MX-1 mixer may be disassembled for cleaning or to change the mixer capacity to accommodate the flow rate. Cleaning may be required if there is erratic gradient performance.

Both mixers consist of the mixer base and barrel, magnetic stir bar, screws, mixer top, and sealing O-rings.

Note: If the system has been used, make sure that any hazardous material has been flushed from the system, the pumps are not running, and any residual pressure has been bled from the system. Drain fluid from the mixer, disconnect any plumbing connections, disconnect the cable from the workstation, and then remove the mixer from the system rack.

1. Use the hex key provided (or any standard 5/32” hex key) to remove the four assembly screws from the top of the mixer.

2. Remove the mixer top and turn it upside down to remove the O-ring. If the O-ring does not easily dislodge, then use your fingers to remove it. Note the position of the O-rings (refer to Figure 11-6).

3. Wipe the inside of the mixer body with a paper towel. To clean the mixer barrel or mixer barrel extension, the O-rings, and the magnetic stir bar, soak or sonicate in a bath containing a mild detergent. Replace damaged O-rings.

   Note: The stir bar is magnetized. To remove it, turn the mixer base upside down and tap it on your hand.

4. Reassemble the mixer. To re-insert the magnetic stir bar, place it on a tabletop and carefully place the mixer base over it.

5. Insert a 1/4-28 plug into one inlet and a 10 ml syringe into the other inlet port and flush the system with DI water or mild detergent solution. Reverse the location of the plug and syringe and repeat.

4. Refer to Figure 11-6 for re-assembling the mixer. Make sure that the magnetic stir bar lies flat and that the O-ring groove in either the mixer barrel or the mixer barrel extender faces up.

   Place an O-ring in each O-ring groove. If you are using only the mixer body, then only one O-ring is required. If either the mixer barrel or the mixer barrel extender is used, then two O-rings are required.

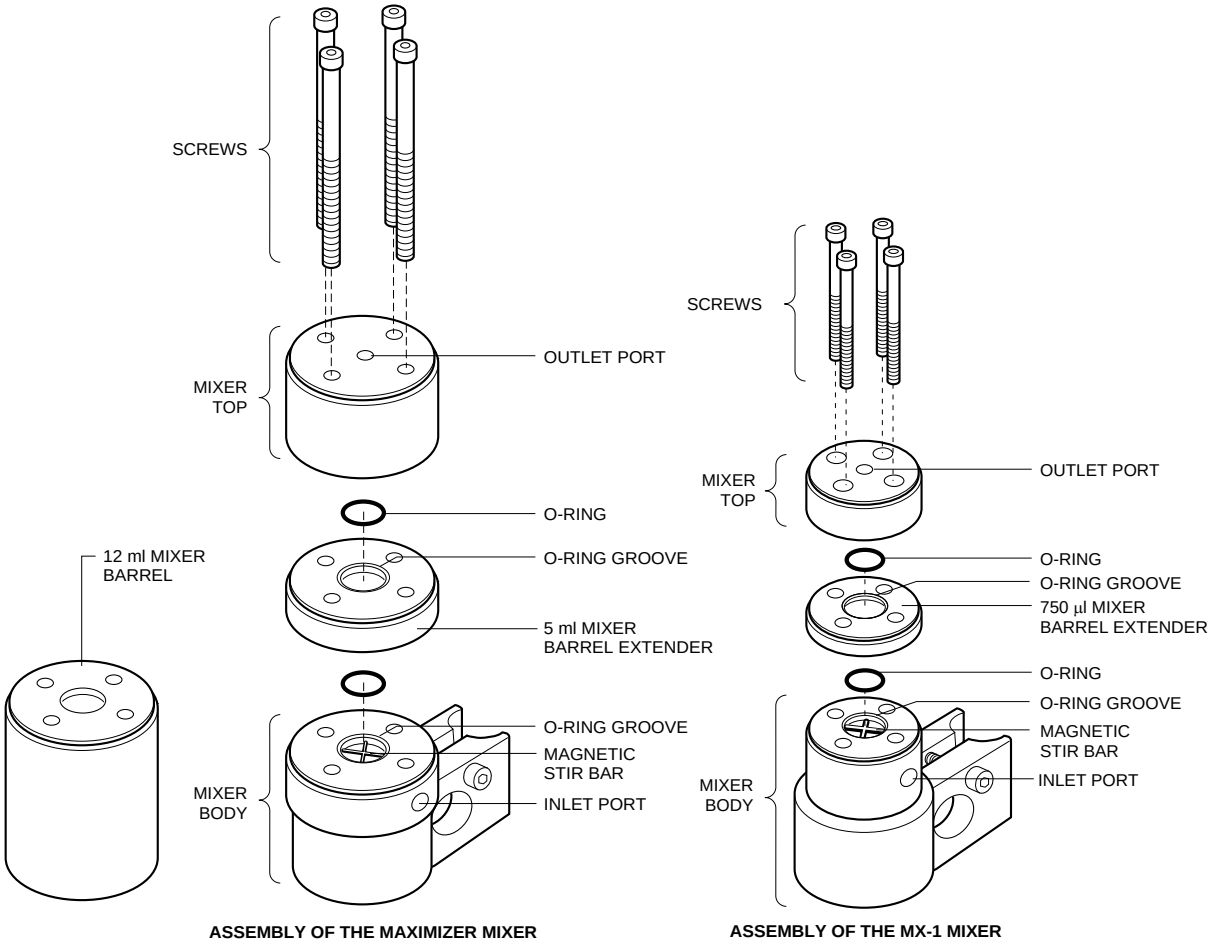


Figure 11-6. Mixer Assembly

11.6 VALVES

The following sections discuss the procedures for cleaning and repair of the SVT3-2, AVR7-3 and AVR9-8 valves.

11.6.1 SVT3-2 Diverter Valve

The SVT3-2 can be disassembled for cleaning or to replace parts which may become damaged over time. Indications of damage include valve leaks and valve switching problems. Items labeled in bold in Figure 11-7 are included in the SVT3-2 Valve Rebuild kit, catalog #760-0411. You will need a #1 Phillips (cross-head) screwdriver and a #0 Phillips (cross-head) screwdriver.

Note: These parts are rather small. Be sure you are working on a clean tabletop with room to lay out these parts and that your hands are clean.

Safety Note: Before disassembling the valve, be sure to flush any hazardous material from the valve and to disconnect it from the Workstation. Wear protective clothing, as appropriate.

To disassemble the valve, follow the procedure below:

1. Remove the large screw that attaches the clamp to the rack.
2. Use the #1 Phillips (cross-head) screwdriver to remove the two screws that attach the clamp to the valve. Set the clamp and screws aside.

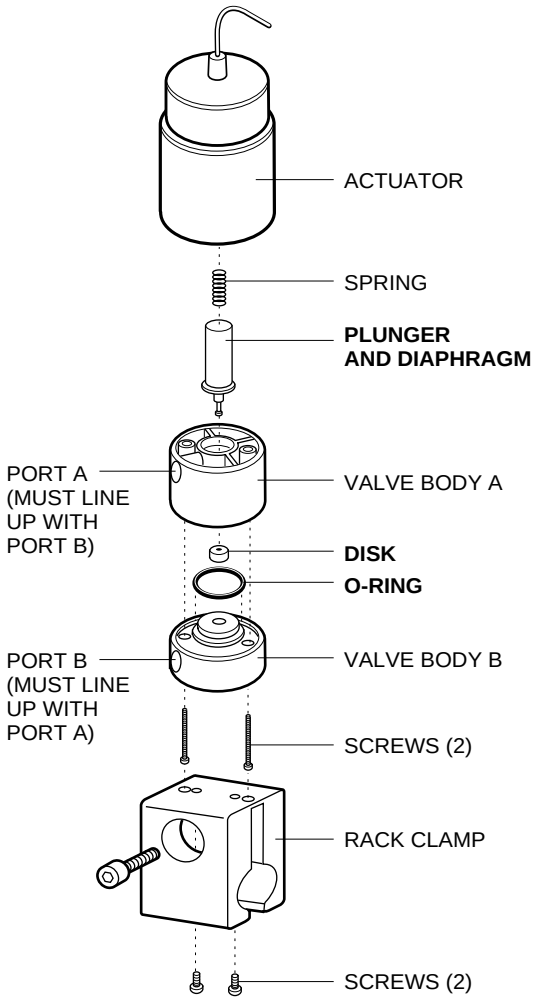


Figure 11-7. SVT3-2 Valve Assembly

3. Use the #0 Phillips (cross-head) screwdriver to remove the two screws and washers that secure valve bodies A and B to the actuator. Set the actuator aside, taking care not to lose the spring.

4. Use your fingers to remove the plunger/diaphragm assembly from the valve body.

5. Remove the label. A new label is provided in the rebuild kit. Note that ports A and B are on one side; the pump/common port is on the other side of valve body A. When reassembled, the new label will not fit properly if ports A & B are not adjacent to each other.

6. Carefully separate the two valve bodies. Note the location of the O-ring.

7. Inspect the disassembled valve. Make sure there are no scratches or foreign material on the sealing surfaces of the two valve bodies. Clean the valve bodies, if necessary, by soaking or sonicating in a bath containing a mild detergent. Replace all required parts, if necessary.

To reassemble the valve:

1. Insert the plunger/diaphragm assembly into valve body A. Invert it and install the sealing disk onto the plunger tip. Make sure the disk inserts into the plunger tip.

2. Install the O-ring on valve body B. Replacing the O-ring is easily done with your finger.

3. Put the two valve bodies together so that the two ports are next to each other. The two valve bodies must lie flush against each other, with no space in between them. Place the two larger screws (removed in step 3 above) through the valve assembly. Set this aside.

4. Set the actuator with its open end facing up.

5. Place the spring in the opening at the center of the actuator so that it fits into the deepest part of the opening. This may take a couple of tries.

6. Place the valve body assembly onto the actuator (the orientation is not specific), then tighten the two screws.

7. Attach the clamp to the valve body with the remaining screws.

8. Apply the valve label. Make sure that the hole in the label matches with the Pump/Common port and does not overlap it.

11.6.2 AVR7-3 and AVR9-8 Valves

The AVR7-3 and AVR9-8 valves are disassembled and cleaned in the same way. The replaceable parts in the valve are the rotor seal and the stator face assembly; these items are included in the AVR7-3 Rebuild kit (catalog #760-0401) and the AVR9-8 Rebuild kit (catalog #760-0403). Indications of damage include valve leaks and valve switching problems.

> **Safety Note:** Before disassembling the valve, be sure to flush any hazardous material from the valve and to disconnect it from the Workstation. Wear protective clothing, as appropriate.

To disassemble the valve, follow the procedure below:

1. With the hex key that is provided, remove the three stator screws.

2. Lift the stator body and stator face assembly from the stator ring. (The stator face assembly usually stays with the stator body when it is lifted off.)

3. Remove the stator face assembly with your fingers and set it aside.

4. Remove the rotor seal by placing a small flat blade screwdriver into the slot on the side of the stator ring. Gently pry up the rotor seal, which will pop off.

5. Inspect the components from the disassembled valve. Make sure there are no scratches or foreign material on the sealing surfaces of the valve. Clean as necessary. Soak or sonicate in a bath containing a mild detergent.

To reassemble the valve:

1. Place the new rotor seal (slot-side facing the stator body), onto the three seal pins of the stator ring. It will only fit one way. Press the rotor seal so that it fits firmly.

2. Place the new stator face assembly onto the stator body so that the pins slip into the mating holes in the stator body. It will only fit one way.

3. Replace the stator body and stator face assembly so that the stator ring enters the mating hole on the stator body.

4. Using the three screws, re-assemble the valve. Tighten each screw equally.

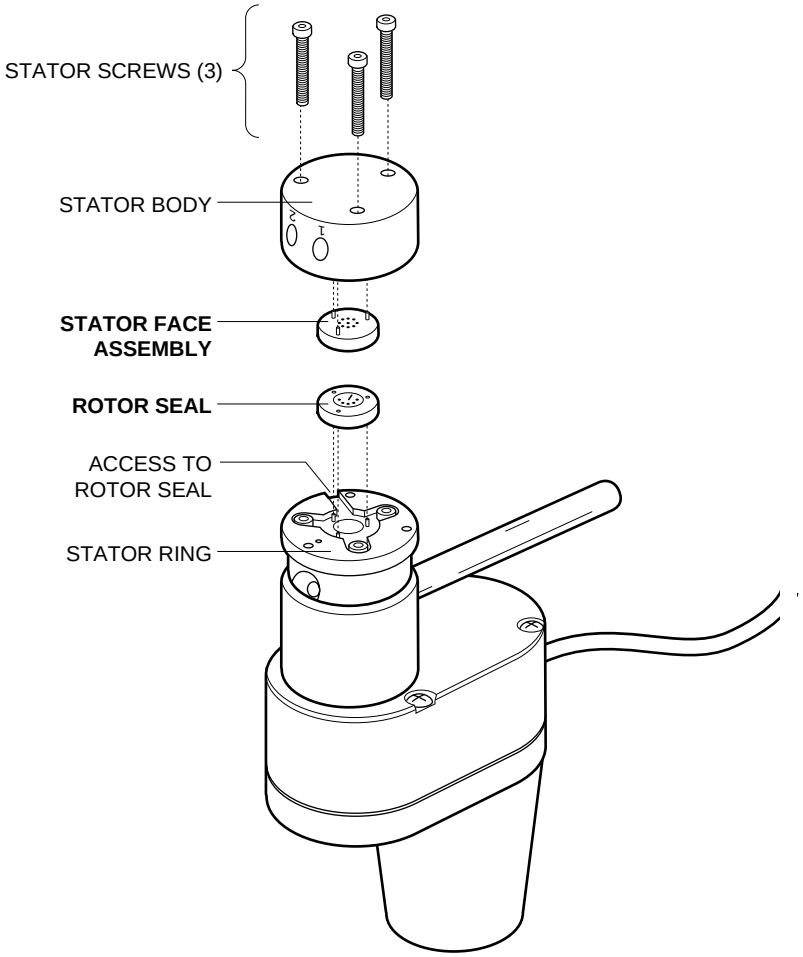


Figure 11-8. AVR7-3 and AVR9-8 Valve Assembly

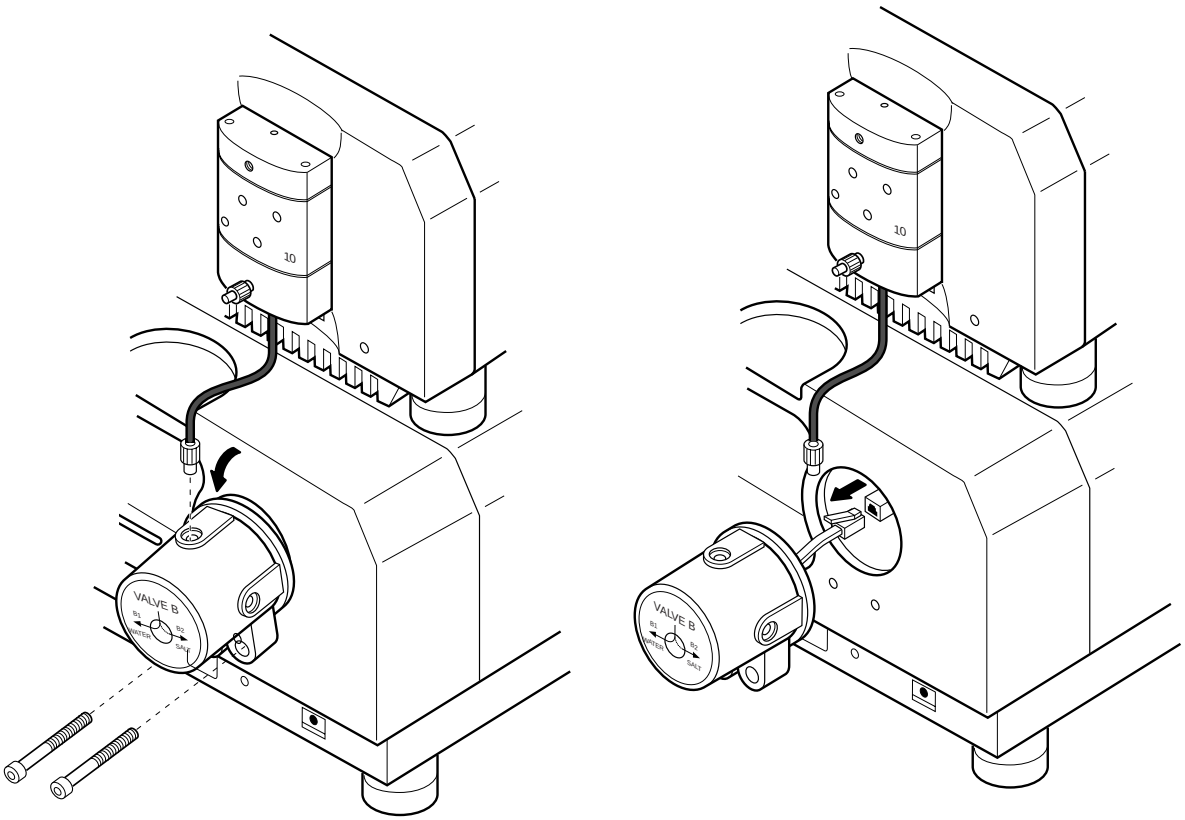
11.7 MAXIMIZER VALVES

Over time, the Maximizer valves may become worn and require replacement. Indications of valve damage include valve leaks and valve switching problems. To replace a valve, you'll need a standard 9/64" hex key; one has been provided for this purpose.

> **Safety Note:** Before removing a valve, be sure to flush any hazardous material from the valve. Wear protective clothing, as appropriate.

To remove the valve, follow the procedure below and refer to Figure 11-9:

1. Use the 9/64" hex key to remove the two screws that attach the valve to the Maximizer. Set the screws aside.
2. Lower the front of the valve to disengage the outlet tubing from the top of the valve.
3. Slowly pull away the valve to expose the wiring behind the valve.
4. Using your fingers, press the connector latch to separate the valve wiring from the Maximizer wiring.



REMOVE MAXIMIZER VALVE DISCONNECT MAXIMIZER VALVE

Figure 11-9. Replacing a Maximizer Valve

To attach the new valve, follow the procedure below:

1. Connect the valve wiring to the Maximizer wiring.
2. Place the valve against the Maximizer, but **do not** reattach the screws at this time. Rotate the valve slightly downward until you can engage the valve with the output tubing to the Workstation. Reattach the output tubing using its fittings.
3. Reattach the valve mounting screws.

12.0 TROUBLESHOOTING DUOFLOW SYSTEMS

The following section lists potential problems and some suggested solutions for the different instruments in the system. You can obtain more information about your system by registering for Consult Bio-Rad at www.biorad.com, where an online technical support service offers an extensive database of frequently asked questions (FAQs).

12.1 TROUBLESHOOTING THE DUOFLOW CONTROLLER AND SOFTWARE

Problem	Possible Cause	Solution
Software response is slow.	Database size may need to be reduced.	1. Archive unnecessary methods and runs. a. Open the **Browser** window. b. Use **COPY OUT** to archive seldom used methods and runs. You may archive to a CD ROM, the BioLogic “C” drive, or any other drive selectable from the Drives drop down menu. 2. Use the Windows 2000 **Disk Defragmenter** utility. (Minimize the Duo-Flow application, and then from the Windows Start button, select Programs>Accessories>System Tools>Disk Defragmenter. Follow the online instructions to defragment files on your disk.
A method or run you expect is not displayed in the Browser.	There may be no apparent cause for this. Use the **Reset** button in the Browser toolbar.	1. From the Browser toolbar, click on the **Reset** button, and then check the database tree for your method or run. **Note:** Using **Reset** collapses the database tree.
While in offline mode, the offline window is not displayed.	The Windows® 2000 minimize button may have been selected.	1. Use any of the following three means of activating the offline window: a. Use the Windows drop-down menu. b. Use the Windows® 2000 taskbar. c. Simultaneously hold down the Alt-Tab keys. 2. With the cursor over the DuoFlow offline button at the bottom of the screen, click on the right mouse button and from the menu that is displayed, select **Restore.**
Problems using Browser functions.	If you are using the right mouse button rather than the toolbar buttons, make sure you have first used the left mouse button to highlight the user, project, method, or run name.	1. Using the left mouse button, click on a user, project, method, or run name. 2. Using the right mouse button, select a function from the drop-down menu that is displayed.

Problem	Possible Cause	Solution
Instrument face-plates (eg. pump, UV, fraction collector) “drop-off” the screen when in Manual mode.	A communication problem has occurred.	1. Exit the DuoFlow application and turn off the Controller. Turn off the Workstation. 2. Make sure all Instrument Bus (phone jack style) connections are secure in all the instruments. Turn the Controller and Workstation back on. 3. If the problem persists, contact Bio-Rad Technical Service.
Controller monitor displays unusual colors or characters.	The monitor or the Controller video card may be defective and need replacement.	1. Exit the DuoFlow application and restart the Controller. 2. If the problem persists, contact Bio-Rad Technical Service.
No sound from monitor speakers.	Cables not connected properly. “Mute” switch is activated.	1. Consult the monitor instruction manual to ensure that the cables are correctly connected. 2. Press the “mute” button, which is located on the front of the monitor to turn on the speakers.
Controller displays “General Protection Fault” message or “locks up” during normal operation.	A communication problem has occured.	1. Exit the DuoFlow application and restart the Controller. 2. If the problem persists, contact Bio-Rad Technical Service.
BioFrac Fraction collector will not go into system mode	The BioFrac is not in Local mode and in the main screen.	Placed the BioFrac in Local mode so that it displays the main screen (start-up screen). Press the System option in the BioFrac panel of the BioLogic software's manual screen.
A method or run is colored red in the browser and I cannot open it.	The file is open in either the BioLogic Online or Offline window. The BioLogic Hardware server is hung.	Go to the BioLogic Online or Offline window where it is open and from the File menu select File/Close. Exit the BioLogic software, press Alt-Ctrl-Del and then select the "Task List..." button. Select the Processes tab and kill any of the following processes that may be running: BL_HWSRV.exe, biologic.exe, _biolog_offline. Restart the BioLogic software.

12.2 TROUBLESHOOTING THE DUOFLOW WORKSTATION PUMP

Problem	Possible Cause	Solution
Pump is not delivering the correct flow rate.	The pumps may not be calibrated. The incorrect pumpheads may be mounted, or the BioLogic Configuration software utility may not have been run after the pumpheads were changed.	1. Recalibrate the pumps. Select Gradient Pump Calibration from the Utilities dropdown menu. 2. Make sure the correct pumpheads (labeled F10 or F40 on the lower-right corner of the center block) are mounted. Then run the BioLogic Configuration software utility and select the correct pumphead.
	There may be problems with the fittings or tubing sizes. It is important to use the wide bore 1/8” OD tubing for the pump inlets and from all pre-pump buffer selection valves to the buffer containers.	3. Check pumps A & B and pre-pump valves. a. Make sure the inlet lines into pumps A and B are tight. b. If pre-pump valves are used, e.g., for buffer selection, make sure all tubing connections are secure and any unused ports are plugged.
	The pump may not be receiving buffer.	4. Check that there is buffer flowing to the pump. a. Ensure that the pump inlet lines are immersed in buffer. b. Ensure that the buffer bottles are positioned at or above the level of the Workstation. c. Ensure all inlet fittings are secure. d. Ensure that any inlet filters are clean. Remove temporarily to test for plugged filters.
	There may be air bubbles trapped in the pumpheads, causing erratic liquid delivery. **Note:** Always degas buffers before use.	5. Re-prime the pumps and purge the lines. a. Re-prime the pumps and ensure that the priming port is shut tight. b. Make sure the Inject Valve is set to Purge position and purge all lines. Refer to the Maintenance section on how to prime the pump and remove trapped air bubbles. To minimize the problem of air bubbles, degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum.

Problem	Possible Cause	Solution
Pump is not delivering the correct flow rate (continued)	Check valves are fouled and should be replaced. The check valves should be replaced on a regular basis, depending on the usage and type of solution or sample pumped through the pumphead. Refer to the Maintenance section for more information on how to replace a check valve.	5. Clean (or replace) the check valves (see Section 11.3.3, Routine Maintenance of the Workstation Pumps.) a. **Cleaning:** Sonicate check valves in a warm detergent solution, rinse with DI water, and re-install. **Replacing:** Replace badly clogged or damaged check valves. b. Recalibrate the pumps after cleaning/replacing check valves. Select **Gradient Pump Calibration** from the **Utilities** drop-down menu.
Liquid is continuously leaking from the pumphead washout drain trough.	The pump's piston seals are worn or damaged.	1. Replace the piston seals on both pumpheads. Clean pistons and piston guides with water, followed by methanol as a precautionary maintenance step. 2. Recalibrate the pumps after changing seals by selecting **Gradient Pump Calibration** from the **Utilities** drop-down menu.
Erratic or reduced flow rate when pumping onto columns which produce low back-pressures (eg., Econo-Pac® cartridges or small, agarose gel affinity columns).	Columns packed with soft gels or large particles typically pro duce very low back-pressures when run at low flow rates.	1. Insert the backpressure regulator between the pump and the injection valve. **CAUTION: Low-pressure columns may burst if the backpressure regulator is placed after the column.**
Loud or unusual noise coming from the front of the workstation.	Residue buildup behind piston seals.	1. The pistons should be rinsed on a nightly basis. This may easily be done automatically by programming a water rinse step at the end of each protocol. This may also be done manually by washing the pistons with the syringe provided in the Fittings kit.
	Sticky pistons.	2. Follow the maintenance procedure to carefully remove and disassemble the pumpheads. Wash the pistons thoroughly with water to remov debris or salt crystals. If the piston seals have many hours of use, they should be replaced following the information in the maintenance section. Ater re-assembly of the pumpheads, pump 100 ml of 100% methanol (MeOH) through each pumphead at 5 ml/min with the 40 psi backpressure regulator in place.

Problem	Possible Cause	Solution
Delivery of liquid is erratic and the pressure readout on the Controller status bar is fluctuating more than 20%.	Severe fluctuation in the back-pressure readout indicates an air bubble trapped in one or both of the pumpheads. **Note:** Always degas buffers before use.	1. Put the AVR7-3 valve into the purge position and purge the system by pressing the Purge buttons on the front panel of the Workstation. 2. Stop or Pause the pumps and re-prime. Loosen the top fitting on the pumphead and force any trapped air out of the pumphead by squirting buffer through the priming port using a syringe. Refer to the maintenance section on how to prime the Workstation pump and remove trapped air bubbles. Always use degassed buffers.
		3. To minimize this problem, use degassed buffers and solutions. Degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum.
	The pump's check valves may need cleaning or replacement.	4. Clean (or replace) the check valves (see section 11.3.3, Routine Maintenance of the Workstation Pump.) a. **Cleaning:** Sonicate check valves in a warm detergent solution, rinse with deionizied (DI) water, and re-install. **Replacing:** Replace badly clogged or damaged check valves. b. Recalibrate the pumps after cleaning/replacing check valves. Select **Gradient Pump Calibration** from the **Utilities** drop-down menu.
The backpressure displayed on the Controller status bar drops to zero or is much lower than expected.	Flow rate may have been changed.	1. Confirm that the flow rate setting has not been changed.
	There may be a tubing or fitting leak.	2. Re-inspect all tubing connections, especially at the pump inlet.
	Pump seals may need replacement.	3. Replace pump seals. A build-up of crystallized buffer salts on the rear of the pumphead is a clear sign for seal replacement. (see Section 11.3.3, Routine Maintenance of the Workstation Pumps.)
	Check valves may need cleaning or replacement.	4. Clean or replace check valves. (see Section 11.3.3, Routine Maintenance of the Workstation Pumps.)

Problem	Possible Cause	Solution
No pressure (psi) reading on Controller status bar or psi value always reads zero.	Pump may not be running, or inlet lines are not primed, or there is a problem in the buffer flow.	1. Check that the pumps are running, and that the inlet lines are primed and not pulled out of the buffer reservoirs. Check for leaking fittings. 2. Check that the buffer flow is going to the column and not to waste via the injection valve purge position.
	The "zero pump pressure" calibration routine may have been used while the system was under pressure.	3. Stop the pump and undo the line from the pump to the valve such that the pressure transducer is definitely at zero psi. Select **Gradient Pump Calibration** from the **Utilities** drop-down menu and perform a **Zero psi** procedure.
Incorrect backpressure readings for a given column.	Flow rate may have been changed. Column may need cleaning and/or a frit replacement. The pressure transducer calibration may be incorrect.	1. Check that the pump is set to deliver the correct flow rate. 2. Consult the column's instruction manual for cleaning procedure. Alternatively, the system tubing may have an obstruction, so inspect the tubing path. 3. Stop the pump, momentarily loosen the pump outlet fitting to release pressure. Select **Gradient Pump Calibration** from the **Utilities** drop-down menu and perform a **Zero psi** procedure.
High backpressure is shutting down the pumps.	Maximum pressure for the pumps is: F10: 3500 psi F40: 1000 psi. The pressure limit settings in the Manual screen (see Figure 7-2) have been set too low. Pressure increases are due to a build-up of particulate matter in the system, causing a resistance to flow. An increase in flow rate also increases the pressure, so be sure to check this setting.	1. Remove the column and run the pumps to determine if the high backpressure is due to the column or not. If the column is suspect, the column may need cleaning along with a frit replacement. Consult the column's instruction manual for cleaning procedure. 2. Alternatively, the system tubing may have an obstruction, so inspect the tubing path. To isolate the blockage, start by loosening connection fittings at the detector and work backwards towards the column and pumphead. 3. Prevent or minimize high backpressure problems by filtering buffers and samples and by changing the pump seals before they deteriorate completely.

12.3 TROUBLESHOOTING THE UV DETECTOR AND UV TRACE

Problem	Possible Cause	Solution
UV baseline is unstable or noisy. UV baseline shows a reproducible zig-zag or saw-tooth trace.	Air bubbles trapped in the analytical 5 mm Z cell are the most common cause of noisy UV baselines. **Note:** Always degas buffers before use.	1. Degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum. 2. Plumb the Z cell with the column outlet connected to the bottom of the flow cell. This will force air bubbles to rise to the top of the flow cell and dissipate. 3. Use of the 40 psi backpressure regulator supplied with the DuoFlow system will remove most air bubbles. Place it directly after the conductivity flow cell and run buffer for several minutes. If the column being used cannot be run with the backpressure regulator and air bubbles are a continuing problem (for example, during some affinity chromatography steps), use the 2mm path length cuvette cell. The straight line flow of the cuvette cell makes bubble removal easier.
Excessive pump pulsations exhibited as regular noise on the baseline.	Air bubbles trapped in the pumpheads can produce exaggerated pulsations which appear as a noisy baseline.	1. Purge the pumpheads to remove the bubble. Refer to Section 11.3.1, Priming the Workstation Pump and Removing Trapped Air Bubbles.
Baseline noise continues when pumps are turned off.	Noise spikes can also be caused by external environmental influences.	1. If the spikes occur at regular intervals (for example, once every 20-30 sec), check for the presence of heating baths, drying ovens, or other heating devices on the same electrical circuit or in close proximity to the DuoFlow system. Turn off these devices to see if the problem goes away.

Problem	Possible Cause	Solution
UV trace will not zero.	If no air bubbles are present in the flow cell, check that the cell itself is clean both internally and externally.	1. Clean the interior of the cell by passing 1M sodium hydroxide (NaOH), water, methanol and water through the cell. Dry the interior of the cell with a stream of high purity nitrogen gas (never use “house” compressed air as it may contain oil droplets). 2. Check for leakage of liquid from the top of the optics unit onto the outside quartz windows of the flow cell. Clean the windows with a soft, non-abrasive damp cloth. 3. Condensation may occur on the exterior windows of the flow cell when moving the optics unit from the lab to a cold room and vice versa. Always allow an equilibration period to compensate for such temperature effects.
Drifting baseline.	Non-homogeneous eluant perhaps due to poor mixing or flow rate variation.	1. Check flow rates of both pumps; recalibrate if needed by selecting **Gradient Pump Calibration** from the **Utilities** drop-down menu.
	Slow column equilibration.	2. Certain ion-exchangers are slow to re-equilibrate especially if just sanitized. Allow a longer equilibration period.
	If drift is due to a higher absorbance, suspect that UV absorbing material may be leaching from the column.	3. To confirm this, remove the column and run deionizied (DI) water or an queous non-absorbing buffer. If the baseline is stable, then the column is suspect. Consult the column's user manual for cleaning procedures.
Negative peaks.	The sample is applied to the column in a different buffer from that used to equilibrate and elute the column. Refractive index changes may be responsible for the negative peaks.	1. Apply the sample in the same buffer used to equilibrate the column.
	The elution buffer may have a higher UV absorbance than the sample components.	2. Check the elution buffer's UV absorbance.

Problem	Possible Cause	Solution
False or “ghost” peaks.	The injection valve, injection port and loop were not rinsed out between sample injections.	1. It is a good idea to allow a larger volume of injection buffer to pass through the inject valve and loop. For example, if the sample loop contains 100 µl, then program a load/injection volume of 200 µl to completely flush the valve and loop. 2. Always clean large volume dynamic loops, such as the Bio-Rad DynaLoop, between use to avoid cross contamination.
	Strongly retained sample components may still be eluting from a previous run.	3. Use stringent elution conditions or consult the column's user manual for an appropriate wash step to remove such components between runs.

12.4 TROUBLESHOOTING THE CONDUCTIVITY FLOW CELL AND TRACE

Problem	Possible Cause	Solution
No values for buffer conductivity are displayed on the Controller status bar.	There may be a loose or incorrect cable connection.	1. Make sure the cable from the Conductivity flow cell is plugged into the correct connector on the rear of the Workstation and that the pins are not bent.
	Conductivity monitor may need calibration.	2. Use the **Conductivity Flow Cell Constant Calibration** function from the Utilities drop-down menu. Either input the constant value which is shown onthe tag attached to the flow cell or calibrate the flow cell using 0.5M sodium chloride (NaCl). Note that the relationship between the conductivity reading and salt concentration is not linear. The curve will flatten out at higher salt concentrations. 3. If a Maximizer is connected, make sure the Conductivity monitor is plugged into it.

Problem	Possible Cause	Solution
Conductivity trace does not follow exactly the theoretical gradient trace %B.		1. The Conductivity flow cell is typically placed after the UV flow cell. The Conductivity trace will be offset from the theoretical gradient based upon the column and system volume.
Distorted conductivity trace.	Mixer capacity is incorrect	1. Refer to Section 2.4, Mixers, for discussion of mixer capacities.

12.5 TROUBLESHOOTING THE MAXIMIZER BUFFER BLENDING

Problem	Possible Cause	Solution
No values for pH are displayed on the Controller status bar.	There may be a loose or incorrect cable connection.	1. Make sure the cable from the pH monitor is connected to a SIM or Maximizer and that the SIM is connected to the system bus.
Noisy or unstable readings	Air bubble on pH probe membrane	1. Remove air bubble.
Drift	Solution temperature is changing	1. Allow all solutions to come to thermal equilibrium.
Fluctuations observed in pH readings during run	Changes in salt concentrations during a run may cause errors in the observed pH	1. The actual pH is may not be in error but only the observed pH. Collect fractions and measure the pH of the solution using a suitable bench top pH monitor during a run. 2. Ensure that the mixer size is appropriate for the flow rate.
The observed buffer pH does not match the desired pH.	The software pH correction may have been entered incorrectly in the Buffer Blending Setup.	1. Check the pH correction setting and change it if necessary.
	The pH electrode is not calibrated.	2. Recalibrate the pH electrode using standards that span the pH range of the buffer being used. Buffers may be incorrectly prepared.
The pH elecrode will not calibrate	The electrode may be dirty or damaged.	1. Clean the pH electrode as described in the instructions that came with the probe. Replace the pH electrode if necessary.

12.6 TROUBLESHOOTING OTHER BIO-RAD INSTRUMENTS AND DEVICES

For troubleshooting information about other Bio-Rad instruments and devices, such as the BioFrac fraction collector and the QuadTec detector, refer to their separate documentation.

APPENDIX A. SPECIFICATIONS

System Power Requirements: BioLogic DuoFlow Controller, Workstation, and Maximizer

- 100 V ~ 5.3 A
- 230 V ~ 3.5 A
- 50 - 60 Hz

DuoFlow Dell Controller

- Pentium IV® Processor, 20 GB hard drive, 256 MB RAM
- SoundNIC and video 4MB graphics performance accelerator
- Dell M781 multimedia 16” monitor
- 3.5” floppy drive; CD-RW CD-ROM drive
- Windows® 2000 operating system
- Microsoft® PS/2 IntelliMouse™
- DuoFlow software, version 5.0 or higher
- Networkable for printer and file access

Maximizer

- Size: 18.5” x 14.25” x 4.33”; 47 x 36 x 11 cm (L x W x H)
- Weight: 11 lbs; 4.8 kg
- Environmental: Cold room compatible
- Solvent compatibility: 1M sodium hydroxide (NaOH), hydrochloric acid (HCl), and organic acids; 0.1M sulfuric acid; 7M urea and guanide hydrochloric acid; 100% methanol (MeOH), isopropanol (IPA), and acetonitrile; 0.1% triflouracetic acid (TFA); 1% detergents (including sodium dodecyl sulfate (SDS)) and TritonX-100

Workstation pump

- Dual piston, positive displacement solvent pumps, with interchangeable solvent delivery pumpheads (F10 and F40) made of bio-compatible PEEK
- F10 pumpheads: *Without* Maximizer: 3,500 psi (233 bar, 23 MPa) from 0.01 to 10.0 ml/min in 0.01 ml/min increments. *With* Maximizer: 3,500 psi (233 bar, 23 MPa) from 0.5 to 20.0 ml/min in 0.02 ml/min increments.

  Flow accuracy: 0.01 to 10.0 ml/min: 2%

  Flow reproducibility: 0.01 rsd
- F40 pumpheads: *Without* Maximizer: 1,000 psi (66 bar, 6.6 MPa) from 0.5 to 40.0 ml/min in 0.01 ml/min increments. *With* Maximizer: 1,000 psi (66 bar, 6.6 MPa) from 1.0 to 80.0 ml/min in 0.02 ml/min increments

  Flow accuracy: 0.5 to 40.0 ml/min: 2%
- Compositional accuracy: ±1.0%
- High pressure dynamic mixing. MX-1 mixer volume without barrel: 263 µl; mixer without mixer barrel extender: 750 µl; mixer with mixer barrel extender: 2.0 ml. Maximizer mixer volume without barrel: 750 µl; mixer with mixer barrel extender: 5.75 ml; mixer with expansion barrel: 12.75 ml.
- Built-in pressure transducer
- Gradient by software control: 1% increments

USB Bitbus Communicator

- Size: 4.25” x 2.75” x 1.125”; 11 x 7 x 3 cm (L x W x H)
- Weight: 6 oz
- Electrical: 5 VDC output

General

- System fittings: Flangeless 1/4-28 flat-bottom fittings, fingertight nuts and ferrules
- System Tubing for flow rates up to 15 ml/min: 1/16” (1.6 mm) OD, 0.020” (0.51 mm) ID orange PEEK tubing. The approximate volume of 1 cm of tubing is 2 µl.

  System Tubing for flow rates of 15 ml/min and greater: 1/16” (1.6 mm) OD, 0.030” (0.76 mm) ID green PEEK tubing. The approximate volume of 1 cm of tubing is 4.5 µl.

  Pump Inlet Tubing: 1/8” (3.2 mm) OD, 0.062” (1.6 mm) ID PTFE tubing
- Wetted materials: 100% bio-compatible
- Solvent compatibility: 1.0 M sodium hydroxide, hydrochloric acid and organic acids, 1.0 M sulfuric acid, 7M urea and Guanidine hydrochloric acid, 100% ethanol, isopropanol, and acetonitrile, 0.1% trifluoroacetic acid (TFA), 1% detergents (including sodium dodecyl sulfate (SDS), Triton X-100)
- Sound level, Workstation: <70 dBa
- Dimensions, Workstation (approx.): ~35 x 41 x 18 cm (W x D x H); height with 3-tray rack: 76 cm Dimensions, Maximizer (approx.): 36 x 47 x 11 cm (W x D x H)
- Weight (Controller, Workstation, monitor, rack, inject valve and detection modules): 34.5 kg (76 lbs) (approximate). Weight, Maximizer: 4.8 kg.
- Operating temperature (excluding Controller and USB Bitbus Communicator): 4° – 40°C

Detection

- Fixed wavelength, mercury lamp with 280/254 nm filter, auto zero; 0.0001 - 2.0 AUFS detection range
- Analytical (5 mm pathlength, 16 µl total volume) flow cell
- Preparative (2 mm pathlength 30 µl volume) flow cell
- Optional 214 nm detection kit (includes zinc lamp, housing, and 214 nm filter)
- Optional wavelength filters include: 214, 313, 365, 405, 436, and 546 nm
- Conductivity detection (500 mS/cm; 6 µl flow cell; accuracy ± 2% full scale)
- Optional flow-through pH monitor (includes flow cell, pH electrode, and SIM)
- Cold room compatible

Sample Loading

- Fixed sample loops, 100 µl to 5.0 ml; automated injection valve included
- Large volume loading through the Gradient Pump. Optional inlet select valves (SV5-4, AVR9-8) for sanitation and multiple sample use.
- Variable volume sample loading through the DynaLoop sliding-piston sample loop.
- Software control of an auxiliary pump for multiple sample loop fills and large volume dynamic loop filling.

Valve Control

- Up to three low-pressure, automated solenoid valves (SV5-4 and SVT3-2) for stream selection.
- Up to three high-pressure, automated valves (AVR7-3 and AVR9-8) for sample inject, column switching and stream selection.

Fraction Collection

- BioFrac fraction collector (collection by Collect All, Threshold, Collection Windows, Collection Windows + Threshold)
- Model 2110 fraction collector (collection by Collect All; when used with optional SVT3-2 Diverter valve, it also offers collection by Threshold, Collection Windows, Collection Windows + Threshold)

Chart Recorder Control

- Model 1327 Dual pen recorder. Paper feed Start/Stop, Pen Up/Down

Signal Import Module

- Up to two Signal Import Modules for importing analog signals from peripheral chromatography detectors

APPENDIX B. PRESSURE CONVERSION TABLE

Use this table to convert pressure units for the pump.

Table B-1
Pressure Conversion

Multiply the units in the left column by the conversion factors listed.

Pressure	PSI	atm	Kg/cm^2	Torr	kPa	Bar	Inches Hg
PSI	1	0.068	0.0703	51.713	6.8948	0.06895	2.0359
atm	14.696	1	1.0332	760	101.32	1.0133	29.921
Kg/cm^2	14.223	0.9678	1	735.56	98.06	0.9806	28.958
Torr	0.0193	0.00132	0.00136	1	0.1330	0.00133	0.0394
kPa	0.1450	0.00987	0.0102	7.52	1	0.0100	0.2962
Bar	14.5038	0.9869	1.0197	751.88	100	1	29.5300
Inches Hg	0.4912	0.0334	0.0345	25.400	3.376	0.03376	1

Example 1: Convert 521 psi to Kg/cm^2
(521 x 0.0703) or (521 psi = 36.63 Kg/cm^2)

Example 2: Convert 60 Bar to psi
(60 x 14.5038) or (60 Bar = 870 psi)

Example 3: Convert 42.70 Bar to Kg/cm^2
(42.70 x 1.0197) = (42.70 Bar = 43.54 Kg/cm^2)

APPENDIX C. WARRANTY STATEMENT

Bio-Rad Laboratories warrants that every instrument it sells will be free from defects in materials and workmanship when it leaves the factory and that, if such defects appear within the first year following purchase, the defective part(s) will be replaced or the entire unit will be replaced, at Bio-Rad's option, free of any charges to the buyer other than expenses incurred in returning the unit to the factory. Bio-Rad's obligation under this warranty is specifically limited to the aforementioned replacement or repairs. However, the following defects are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or their authorized agent.
3. Use with fittings or other spare parts not specified by Bio-Rad Laboratories.
4. Damage caused by deliberate or accidental misuse.
5. Damage caused by disaster.
6. Damage due to use of improper solvent or sample.
7. Tubing, fittings, lamps, pistons, check valves, piston seals, and other consumables.

"The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties, of merchantability, fitness for a particular purpose or otherwise, expressed or implied in fact or by law, and state Bio-Rad's entire and exclusive liability and Buyer's exclusive remedy for any claims or damages in connection with the furnishing of goods or parts, their design, suitability for use, installation or operation, Bio-Rad will in no event be liable for any special, incidental or consequential damages whatsoever, and Bio-Rad's liability under no circumstances will exceed the contract price for the goods for which liability is claimed."

Under this warranty Bio-Rad's obligation with respect to transportation expenses is limited to the cost of shipping the repaired or replacement instrument to the buyer, provided that such repair or replacement comes within the terms of this warranty.

For additional help, contact your local Bio-Rad representative. In the United States, call Technical Service at 1-800-4BIORAD.

APPENDIX D ORDERING INFORMATION

760-0180 F40 Pump Kit. Includes fully assembled F40 pumpheads with check valves and seals installed, four F40 piston assemblies, one mixer barrel extender, two mixer O-rings, a 2mm UV flow cell, PEEK tubing 0.030" (0.76 mm) ID, and necessary tools.
760-0110 F10 Pump Kit. Includes fully assembled F10 pumpheads with check valves and seals installed and four F10 piston assemblies.
760-0164 F10 Pump Maintenance Kit, services one F10 pumphead. Kit includes two piston seals, four check valves, seal removal tool, and two O-rings.
760-0161 F10 Piston Seals (2) and Seal Tool. Two piston seals required to service one F10 pump.
760-0162 F10 Piston Assembly (2). Two piston assemblies required to service one F10 pump.
760-0184 F40 Pump Maintenance Kit, services one 40ml pump. Kit includes two piston seals, two check valves, and two pistons.
760-0172 F40 Piston Seals (2) and Seal Tool. Two piston seals required to service one F40 pump.
750-0162 Check Valve (1). Four check valves required to service one pump; for F10 and F40 pumps.
750-0111 EZ Logic Integration Software Package.
760-2030 USB Bitbus Communicator.

Mixers

760-0170 MX-1 Mixer. Mixer includes mixer body (263µl) and expansion barrel (750µl).
760-0171 MX-1 Mixer Barrel Extender (2ml).
100-1627 MX-1 Mixer Service Kit.
920-8529 MX-1 Mixer Barrel Extender (1) (750µl).
760-2010 Maximizer Mixer. Includes mixer body (750µl), 5ml and 12ml expansion barrels, five O-rings, stir bar, and installation screws for all barrel sizes.
760-2005 Maximizer Mixer Barrel Extender (5ml).
760-2012 Maximizer Mixer Barrel Extender (12ml).

Detectors

760-1300 QuadTec DuoFlow Detector Kit 100/110, 200/240 V.
Includes QuadTec Detector, 3mm flow cell, System Cable 25 QuadTec RS232 cable, 4x10-32 Fingertight fittings, Instrument Control Module (ICM), System Cable 26 ICM power cable, System Cable 17 instrument bus cable, QuadTec Instruction Manual, US power cord.
760-1330 QuadTec Deuterium Lamp.
760-1331 QuadTec Halogen Lamp and Holder. The holder is required for first time use.
760-1332 QuadTec Halogen Lamp.
760-1306 QuadTec Flow Cell, PEEK 3mm path length.
760-1311 QuadTec 4x10-32 Long fittings for 3mm flow cell.
760-1406 QuadTec Flow Cell, PEEK High speed for 80 ml/min flow rates.
760-1408 QuadTec 4x10-32 Long fittings for Hi speed flow cell.
760-1301 QuadTec Instruction Manual.
750-0200 UV Detector Kit. Includes UV Detector with 254/280nm filter, 5mm flow cell, conductivity flowcell, and backpressure regulator.
750-0214 214 nm Conversion Kit (Zinc lamp, housing, 214 nm filter) for Model OM-II UV Detector optics modules beginning with serial number 362 BR.
750-0215 214 nm Conversion Kit (Zinc lamp, housing, 214 nm filter) for Model OM-IO UV Detector optics modules, beginning with serial number 345 BR and prior to 362 BR.
750-0210 UV Detector Flow Cell, 2 mm path length.
750-0212 UV Detector Flow Cell, 5 mm path length.
750-0216 Mercury Lamp, replacement.
750-0217 Zinc Lamp (214 nm), replacement.
750-0220 Filter, 280 and 254 nm.
750-0221 Filter, 214 nm.

750-0223 Filter, 365 nm.
750-0224 Filter, 405 nm.
750-0225 Filter, 436 nm.
750-0226 Filter, 546 nm.
750-0240 Conductivity Flow Cell, replacement.
750-0230 Backpressure regulator.
750-0502 Signal Import Module (SIM).

Valves

760-2008 Maximizer Proportioning Valve.
760-0406 AVR7-3 Sample Injection Valve.
760-0401 AVR7-3 Valve Rebuild Kit.
750-0471 AVR7-3 Sample Injection Port.
760-0408 AVR9-8 Stream Select Valve.
760-0403 AVR9-8 Valve Rebuild Kit.
760-0410 SVT3-2 Diverter Valve.
760-0411 SVT3-2 Valve Rebuild Kit.
750-0415 SV5-4 Select Valve.
125-0224 Inject Needle, 22-gauge blunt, fits sample injection port.

750-0251 Rack

750-0268 Rack expansion kit (two trays, two vertical bars, sixteen sleeves).
750-0260 Column clamp set.
750-0261 Rack tray; includes eight sleeves, one drain/plug.
750-0262 Vertical bar, long (2).
750-0263 Vertical bar, short (2).
750-0264 Horizontal bar kit (two tie bars, four bar clamps).
750-0265 Bar clamps (5).
750-0266 Cable Manager clips (4).
750-0269 System wrench set.

pH Monitor

760-2040 DuoFlow pH Monitor.
760-2042 pH Electrode, replacement.
760-2044 Flow Cell, replacement.
750-0502 Signal Import Module (SIM).
910-1828 O-ring, pH Flow Cell, size 2-009.
910-3014 O-ring, pH Flow Cell, size 2-018.
760-2046 BioLogic pH Monitor Tubing Kit.

Sample Injection Loops

750-0490 Small Volume Sample Loop Kit (100, 250, and 500µl loops). Nominal pressure limit of 4000 psi.
750-0491 Large Volume Sample Loop Kit (1, 2, and 5ml loops). Nominal pressure limit of 3000 psi.
750-0492 100 µl injection loop. Nominal pressure limit of 4000 psi.
750-0493 250 µl injection loop. Nominal pressure limit of 4000 psi.
750-0494 500 µl injection loop. Nominal pressure limit of 4000 psi.
750-0495 1 ml injection loop. Nominal pressure limit of 3000 psi.
750-0496 2 ml injection loop. Nominal pressure limit of 3000 psi.
750-0497 5 ml injection loop. Nominal pressure limit of 3000 psi.
750-0451 DynaLoop 25, for 25ml samples
750-0452 DynaLoop 90, for 90ml samples

Fittings

760-0550 DuoFlow Fittings Kit.
750-0560 DuoFlow Fittings Tool.
750-0553 1/8” (3.2 mm) Pre-pump Fittings (includes nut, ferrule, and lock ring) (10).
750-0554 1/16” (1.6 mm) Post-pump Fittings (includes nut, ferrule and lock ring) (10).
750-0570 1/8” (3.2 mm) Nut (5).
750-0571 1/8” (3.2 mm) ferrule and lock ring (5).
750-0569 1/16” (1.6 mm) Nut (10).
750-0556 1/16” (1.6 mm) ferrule and lock ring (10).
750-0703 Inline Filter Kit. Includes one inline filter, two replacement Ultra High Molecular Weight PolyEthylene (UHMWPE) filter frits.
750-0704 Filter frits, replacement (5).
750-0566 Bottle Cap Kit, (includes two bottle caps, two cap plugs).
750-0559 Caps (5 pack).
750-0561 1/4-28 to M6 (FPLC column adapter) (2).
750-0562 1/4-28 to 1/4-28 union (5).
750-0563 1/4-28 plug (5).
750-0564 HPLC Column to DuoFlow System Fittings Kit (1 set).
750-0565 Econo-Column to DuoFlow System Fittings Kit (1 set).
732-0113 Econo-Pac® Cartridge to DuoFlow System Fittings Kit (1 set).
750-0471 Econo-Column® to DuoFlow System Fittings Kit (1 set).

Tubing

750-0603 PTFE tubing, 1/8” (3.2 mm) OD x 0.062” (1.6 mm) ID x 15 feet (4.6 m), for pre-pump connections.
760-0604 PEEK tubing, 1/16” (1.6 mm) OD x 0.020” (0.51 mm) ID x 30 feet (9.2 m), for flow rates less than or equal to 10ml/min.
760-0605 PEEK tubing, 1/16” (1.6 mm) OD x 0.030” (0.76 mm) ID x 30 feet (9.2 m), for flow rates greater than 10ml/min.
760-0650 DuoFlow F10 Tubing Kit. Includes premade tubing for basic installation of DuoFlow with F10 Workstation system.
760-0652 DuoFlow F40 Tubing Kit. Includes premade tubing for basic installation of DuoFlow with F40 Workstation system.
760-2002 Maximizer Tubing Kit. Includes premade tubing for buffer connection.
760-2046 DuoFlow pH Monitor Tubing Kit.

Fraction Collectors

741-0002 BioFrac Kit 110/120V. Includes the base unit, 100V power cord, Fittings kit, Diverter valve, Instruction manual, and Rack set F1 (2 x flatpack, 12-13 mm).
741-0003 BioFrac Kit 220/240V. Includes the base unit, Fittings kit, Diverter valve, Instruction manual, and Rack set F1 (2 x flatpack , 12-13 mm).
741-0008 BioFrac Diverter valve.
741-0010 BioFrac Rack set F1 (2 x flatpack, 10-13mm).
741-0011 BioFrac Rack set F2 (2 x flatpack, 15-16mm).
741-0012 BioFrac Rack set F3 (2 x flatpack, 18-20mm).
741-0013 BioFrac Rack set H1 (4 x flatpack, 1.5 ml microtubes).
741-0014 BioFrac Rack set H2 (4 x flatpack, 0.5 ml microtubes).
741-0015 BioFrac Rack set H3 (4 x flatpack, 16 mm scintillation vials).
741-0016 BioFrac Rack set H4 (4 x flatpack, 30 mm large scintillation vials).
741-0017 BioFrac Ice Bath & Microplate Rack.
741-0018 BioFrac Prep-20 Preparative Rack 741.

731-8238 Model 2128 Diverter Valve.
731-8122 Model 2110 Fraction Collector, 110 V.
731-8120 Model 2110 Fraction Collector, 220 V.
760-0410 Model 2110 Diverter Valve, SVT3-2.
731-8130 Model 2110 Carousel, 80-tube capacity.
731-8135 Model 2110 Micro Tube Adapter.
731-8136 Model 2110 Carousel Dust Cover.

Pumps

731-9001 Econo Gradient Pump, 100/120 V. Includes Tubing and Fittings kit.
731-9002 Econo Gradient Pump, 220/240 V. Includes Tubing and Fittings kit.
731-8140 Model EP-1 Econo Pump, 100/120 V.
731-8142 Model EP-1 Econo Pump, 220/240 V.

Chart Recorder

731-8250 Model 1327 Chart Recorder, with USA, Canada, Japan, Mexico, Taiwan, and Latin America power adapter.
731-8253 Model 1327 Chart Recorder, with UK, Commonwealth power adapter.
731-8254 Model 1327 Chart Recorder, with Australia, New Zealand power adapter.
731-8255 Model 1327 Chart Recorder, with European power adapter.

Cables

750-0650 System Cable 17 (instrument bus communication), 4 feet (1.2 m).
750-0651 System Cable 18 (instrument bus communication), 12 feet (3.7 m).
750-0652 System Cable 19 (instrument bus communication), 30 feet (9.2 m).
750-0655 System Cable 21 (instrument bus communication), 100 feet (30 m).
760-1309 System Cable 24, QuadTec analog to bare wire, includes two cables to connect to two SIMs.
760-1307 System Cable 25, QuadTec RS232, connects QuadTec to ICM.
760-1321 System Cable 26, ICM power cord, connects to DuoFlow Workstation.
760-2004 System Cable 30 (bus communication), 1 foot (30.5 cm).
760-2032 System Cable 31, USB Bitbus Communicator to controller.
731-8262 System Cable 2 (mini-DIN to Model 1327 DIN).
731-8264 System Cable 4 (mini-DIN to rec banana).
731-8265 System Cable 5 (Workstation AUX bare wires to DB-9).
731-8267 System Cable 7 (mini-DIN to bare wires).
750-0653 System Cable 20 (mini-DIN to DIN).

Miscellaneous

760-0135 Starter Kit.
720-0001 UNO Q1 Column.
760-0001 BioLogic DuoFlow Instruction Manual.

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Life Science Group

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