

BIOLOGIC LP CHROMATOGRAPHY SYSTEM

INSTRUCTION MANUAL

Catalog Numbers 731-8300 731-8301



TABLE OF CONTENTS

Safety Getting Started Quickly

•	Introduction	
1.1	Overview	
1.2	Features	
1.3	Unpacking	
1.4	Description of System Components	3
Chapter 2.0	Description of Components	6
2.1	BioLogic LP	
2.2	Proportioning Valve/Mixer Module	
2.3	UV Detector	10
2.4	Conductivity Detector	11
2.5	Bio-Rad System Options	
	2.5.1 Model 2110 Fraction Collector	
	2.5.2 Model 2128 Fraction Collector	13
	2.5.3 Model 1327 Chart Recorder	14
2.6	Non-Bio-Rad System Options	15
	2.6.1 Non-Bio-Rad Fraction Collectors	
	2.6.2 Non-Bio-Rad Chart Recorders	16
Chapter 3.0	System Connections	17
Chapter 4.0	System Plumbing	22
4.1	General Guidelines for Plumbing the System	
4.2	Adjusting the Platen and Plumbing The Peristaltic Pump	24
4.3	Plumbing the System	26
4.4	Purging the System	30
Chapter 5.0	System Operation	31
5.1	Front Panel Controls	
5.2	Manual Mode Operation	34
	5.2.1 Manual Mode Operation of the Pump	34
	5.2.2 Manual Mode Operation of the Fraction Collector	38
	5.2.3 Manual Mode Operation of the Alarms	41
	5.2.4 Manual Mode Operation of the UV Monitor	42
	5.2.5 Manual Mode Operation of the Conductivity Monitor	44
	5.2.6 Manual Mode Operation of the Valves	46
	5.2.7 Manual Mode Operation of the Chart Recorder	48
5.3	Programming Mode	
	5.3.1 Programming Mode's Main Menu	
	5.3.2 Creating a New Method	
	5.3.3 Viewing and Editing a Method	
	5.3.4 Program Mode's Pump Table	
	5.3.5 Program Mode's Fraction Collector Table	
	5.3.5.1 Collect All Mode	64
	5.3.5.2 Threshold Collection Mode	65

				Collection Windows Mode	
			5.3.5.4	Threshold and Collection Windows Mode	70
		5.3.6	Progran	nming Mode's Alarm Table	62
		5.3.7	Entering	Method Names	75
	5.4	Run N	/lode		76
		5.4.1	Starting	a Run	76
			5.4.1.1	Errors Which Prevent the Start of Runs	78
			5.4.1.2	Using the Delay Feature	78
		5.4.2	Run in F	Progress	79
			5.4.2.1	Information Available during a Run	80
			5.4.2.2	Holding a Run	81
			5.4.2.3	Pausing a Run	82
			5.4.2.4	Manual Override	83
		5.4.3	Interpre	ting the Chart Recorder Trace	84
Ch	apter 6.0	Maint	enance a	and Troubleshooting	85
•	6.1			Storage	
				on	
	0.2			l Calibration	
				Alibration	
	6.3			ing Valves, Flow Cells, and Filters	
	0.0			Valves and Flow Cells	
				g Valves and Flow Cells	
				g the UV Optics Module's Filters	
	6.4			pportioning Valve and Mixer	
				Lamp in the UV Optics Module	
	6.6			g	
Αp	pendix A.			S	
				Ordering Information	
•			.,	3	_
	T 05 510				
	T OF FIG				4
				r module	
				nd Separate Holder	
				ector	
				lector	
				der	
	_			ogic LP's Fraction Collector and Chart Recorder Connectors	
				P System	
				ns	
				D. Cantrallaria D	
				P Controller's Pump	
14.	System F	riumbir	ıg	D	27
				Pump	
				Fraction Collector	
				Alarms	
				UV Monitor	
				Conductivity Monitor	
	Contents			Valves	
/	CHIMITE	/۱۱ استان			יור

22.	List of Methods	.52
	Programming a New Method	
	Programming: The Pump Table	
25.	Programming: The Fraction Collector's Summary	63
	Programming: The Fraction Collector's Collect All Mode	
27.	Programming: The Fraction Collector's Threshold Mode	66
28	Programming: The Fraction Collector's Windows Mode	69
	Programming: The Fraction Collector's Threshold with Collection Windows Mode	
	Programming: The Alarm Table	
31.	Run In Progress	79
32.	UV Optics Module	89
	·	
110	T OF TABLES	
1.	Description of System Components	2
2.	BioLogic LP Controller's Front Panel Features	
3.	BioLogic LP Controller's Rear Panel Connectors	
3. 4.	Comparison of Flow Rate Ranges for Different Tubing IDs	
4 . 5.	Front Panel Controls	
5. 6.	Manual Mode Operation: Pump	
7.	Manual Mode Operation: Fraction Collector	
8.	Manual Mode Operation: Alarms	
9.	Manual Mode Operation: UV Monitor	
	Manual Mode Operation: Conductivity Monitor	
	Manual Mode Operation: Valves	
	Manual Mode Operation: Chart Recorder	
	Programming Mode Operation: List of Methods	
	Programming Mode Operation: New Method	
	Programming Mode Operation: Viewing and Editing a Method	
	Programming Mode's Pump Table	
	Program Mode's Fraction Collection Table	
	Program Mode's Fraction Collection Table: Collect All	
	Program Mode's Fraction Collection Table: Threshold Collection	
	Program Mode's Fraction Collection Table: Collection Windows	
	Program Mode's Fraction Collection Table: Threshold and Collection Windows	
	Program Mode's Alarm Table	
	Program Mode: Entering Method Names	
	Run Mode: Starting a Run	
	Run Mode: Run Hold	
	Run Pause	

SAFETY



Caution/Warning

Disconnect power to any BioLogic LP component before servicing. No userserviceable parts are inside any component. Refer servicing to Bio-Rad service personnel.

The Bio-Rad BioLogic LP is certified to meet the I.E. C. 1010* safety standard for safety of laboratory equipment. Certified products are safe to use when operated in accordance with the instruction manual. This safety certification does not extend to other chromatography equipment or accessories not I.E.C. 1010 certified, even when connected to this BioLogic LP system.

This instrument is intended for laboratory use only.

The BioLogic LP conforms to the "Class A" standards for Electromagnetic Emissions, intended for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or on the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

This instrument should not be modified or altered in any way. Alteration of this instrument will void the manufacturer's warranty, void the I.E.C. 1010 certification, and create a potential safety hazard for the user.

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

*I.E.C. 1010 is an internationally accepted electrical safety standard for laboratory instruments.

GETTING STARTED QUICKLY

This manual provides detailed discussion of the use and maintenance of the BioLogic LP system. Listed below is an overview of the steps involved in setting up and using your system.

Setting up the electrical and plumbing connections:

- 1. Connect system cabling, as shown in Figure 11, System Cable Connections. Note the following:
 - a. Chart recorder: Be sure to plug the conductivity signal banana plugs into Channel 2 of the chart recorder. (Refer to Chapter 3, step 6 for connecting the chart recorder.)
 - All chart recorder controls should be set to the position marked in green.
 - b. Fraction collector: Because this depends on the type of fraction collector you will be using, it is recommended that you read Chapter 3, steps 7 and 8 for connecting the fraction collector.
- 2. Select and install the pump head tubing: Select a tubing according to the flow rate that will be used for the separation. (Refer to sections 4.1 and 4.2 for plumbing the pump.)
- 3. Adjust the platen pressure: Read section 4.2, which discusses adjusting the platen.
- 4. Plumb the system: Refer to Figure 14, System Plumbing. Note the following:
 - a. Arrows embossed on the UV Optics module indicate flow direction.
 - b. The MV-6 Inject valve will conform to the diagram when the knob is turned counterclockwise.
- 5. Provide a drain tube for the rack tray.

Setting up the operating conditions:

- 1. Using the BioLogic Lp's front panel, from the Mode keys select **Manual**.
 - a. Select your fraction collector: From the Instrument keys select **Collector**. From the screen display select the **Model** softkey.
 - b. Calibrate the pump: This is essential for acquiring an accurate displayed flow rate. From the Instrument keys select **Pump**. From the screen display select the **Flow** softkey. To calibrate, read section 5.2.1, Manual Mode Operation of the Pump.
 - c. Purge air from the system: From the screen display select the **Purge** softkey.
 - d. When the UV Monitor has warmed up (5 to 10 minutes after power on), select a sensitivity range and zero the UV Monitor: From the Instrument keys select **UV**. From the screen display select the **Set Range** or **Set Max** (your choice) and **Zero** softkeys.
 - Using the thumbwheel on the chart recorder, adjust the position of the Channel 1 chart recorder pen.
 - e. Select a sensitivity range for the Conductivity Monitor: From the Instrument keys select Cond.
 From the screen display select the Set Range or Min/Max (your choice) softkey.
 Using the chart recorder's thumbwheel, adjust the position of the Channel 2 chart recorder pen.
- 2. Program a method: From the Mode keys select **Program**. Refer to section 5.3 for discussion.

- 3. Make final adjustments. From the Mode keys select **Manual**, and from the Instrument keys select **Pump**. Start the pump, and with fluid flowing through the system, check the following:
 - a. Check the range setting for the UV Monitor, zero the UV Monitor, and position the Channel 1 pen on the chart recorder.
 - b. Check the range setting for the Conductivity Monitor, and position the Channel 2 pen on the chart recorder.
 - c. Check that a sufficient number of tubes are in the fraction collector rack.
 - d. Check all fittings for leaks.
 - e. Turn the MV-6 valve know to the left, and load the sample loop.
 - f. Check that the "Waste" tube is in the waste container or drain, and make sure that buffer inlet lines are submerged to the bottom of their containers.
- 4. Run the method: From the Mode keys select **Run** to start the separation. When the run starts, turn the MV-6 Inject valve knob to the right. (Refer to section 5.4.)

1.0 INTRODUCTION

1.1 OVERVIEW

The BioLogic LP low-pressure, gradient chromatography system is designed for the purification of proteins, peptides, and other biomolecules where recovery of biological activity is of primary concern.

The BioLogic LP Controller is microprocessor controlled, with easy-to-use front panel controls and menu-driven software for manual operation, system setup, method editing and run operations.

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.

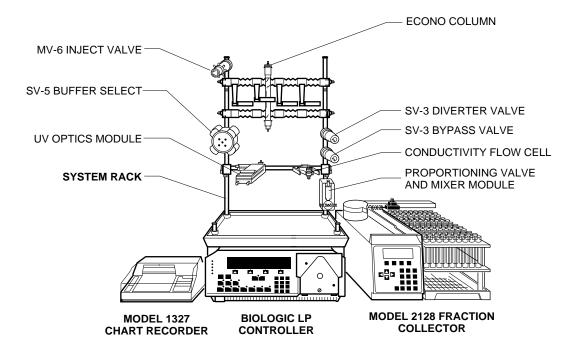


Figure 1. BioLogic LP System

1.2 FEATURES

The BioLogic LP System provides the following features:

- A space-saving modular and stackable design which minimizes the system footprint on the bench.
- Programming separation methods is menu-driven, easy and intuitive using either Time-based or Volume-based steps. Method storage capacity easily meets most laboratory requirements.
- On-screen help function.
- Single-point control of pump, UV and Conductivity monitors, chart recorder, fraction collector, valves, and timer.
- Dynamic mixing for accurate and reproducible gradient formation.
- The use of biocompatible materials throughout the flowpath to ensure maximum recovery of biological activity.
- Operation at 280nm and 254nm, with a preparative UV flow cell
- Standard conductivity cell to monitor gradient formation.
- Four Fraction Collection schemes are standard. Collection is volume-based or time-based with Collect All, Collection Windows, Threshold and Threshold + Collection Window modes. A wide range of fraction collectors may be used, including Bio-Rad's Model 2128 and Model 2110 fraction collectors.
- Software control of diverter, column bypass, and buffer select valves.
- Sufficient tray space on the basic System Rack to hold a Model 2110 Fraction Collector or a Model 1327 Chart Recorder. Additional rack components are available to make a three-tray configuration.
- System Rack includes column supports to eliminate rack clutter.

1.3 UNPACKING

When you receive the BioLogic LP 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 may have occurred, 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.

1.4 DESCRIPTION OF SYSTEM COMPONENTS

The following sections identify the key features of the BioLogic LP System and available options.

Table 1.

Description of System Components

Component	Function
Controller	The BioLogic LP Controller consists of the following:
	 System Software. Through the software's menu-driven interface, each instrument in the system can be operated manually, or as part of a process as specified by the method you create. Software controls the Peristaltic Pump, the Mixer/Proportioning valve, the Valves, the UV/Conductivity Monitors, and peripheral instruments (such as the Model 2128 frac- tion collector and Model 1327 Chart Recorder).
	 Peristaltic Pump. This is a two-channel, bi-directional, variable speed, peristaltic pump, delivering flow rates from 0.05 to 40 ml/min.
	 Control circuitry for the UV and Conductivity Monitors. Signal export ports for UV and Conductivity data at 1V and chart recorder control (pen up/down, start/stop).
	• Control circuitry for the system valves (low pressure solenoid and buffer select valves).
	An output power connector to the UV lamp.

Table 1. (continued) Description of System Components

Component	Function
System Rack	This is the system organizer for columns and cartridges, buffer containers, sample inject and buffer select valves, UV and Conductivity Flow Cells, and other devices used with the system. The optional BioLogic Rack Expansion kit is available to extend the rack to 3 trays.
Proportioning Valve/Mixer Module	This combination of valve and mixer module mixes and proportions two buffers. It is designed to be rack mounted or free-standing.
UV Detector	This consists of the UV Optics Module and the control circuitry within the BioLogic LP Controller. The UV Optics Module includes a mercury lamp and filters for fixed wavelength UV detection at 280nm and 254nm. The flow cell has a path length of 2 mm, an internal volume of 80 μ l, and an illuminated volume of 3 μ l.
Conductivity Detector	This consists of the Conductivity Flow Cell and the control circuitry within the BioLogic LP Controller. The Conductivity Flow Cell provides instantaneous, on-line readings of conductance for monitoring a salt gradient and optimization of column chromatographic conditions.
Valves	Valves for the BioLogic LP include:
•	MV-6 Inject Valve
•	SV-5 Buffer Select valve
•	SV-3 Diverter/Column Bypass valves
Sample Loops	Tygon tubing sample loops are prepared by the user.
Fraction Collector	Fraction collectors are controlled by the BioLogic LP Controller via the Fraction Collector port. Fraction advance marks (event marks) are embedded in the UV signal sent from the Controller to a chart recorder. The following fraction collectors are supported:
•	• The Model 2128 provides X-Y motion drop dispensing. The Model 2128 accommodates a wide range of tube diameters and lengths, microtiter plates, micro-tubes, and "bottle size" fractions. The Model 2128 is ideally suited for both analytical and preparative applications.
•	• The Model 2110 uses a stationary drop-dispensing head to collect up to 80 fractions in a carousel. The SV-3 Diverter valve is required for full operation. The Model 2110 is controlled by the BioLogic LP Controller via the Fraction Collector port.
•	• Generic fraction collectors may be used with the BioLogic LP as long as tube advances can be initiated by a TTL pulse (active high or active low, 100 ms duration.) The SV-3 Diverter valve is required for full operation.
	Regardless of which fraction collector is chosen, fraction time/volume and collection parameters such as Collect All, Collection Windows, Threshold, and Threshold + Collection Windows are controlled from the BioLogic LP Controller.

Table 1. (continued) Description of System Components

Component	Function
Chart recorder	Bio-Rad offers the Model 1327 Chart Recorder as a system option. This is a dual pen chart recorder for Conductivity and UV Detector readings. Stop/Start and Pen Up/Down functions are controlled by the BioLogic LP Controller. Chart speed is set at the recorder itself (the BioLogic LP Controller does not control this function). Event marks including fraction advance marks are embedded in the UV signal sent from the BioLogic LP Controller to the chart recorder.
	The BioLogic LP may be used with any recording instrument (such as a chart recorder, integrator, or data acquisition system) which will accept a 0 to 1 volt analog input.
Tubing	The following tubing is supplied with the unit: System tubing: 0.8mm ID, 1.6mm ID Tygon® tubing. Pump head tubing: 0.8mm ID, 1.6mm ID, 3.2mm ID PharMed® tubing.
Fittings	Luer fittings.

PharMed and **Tygon** are registered trademarks of Norton Co.

2.0 DESCRIPTION OF COMPONENTS

The following sections identify the key features of each of the major system components.

2.1 BIOLOGIC LP

The BioLogic LP Controller is described in Table 2.

Table 2.
BioLogic LP Controller's Front Panel Features

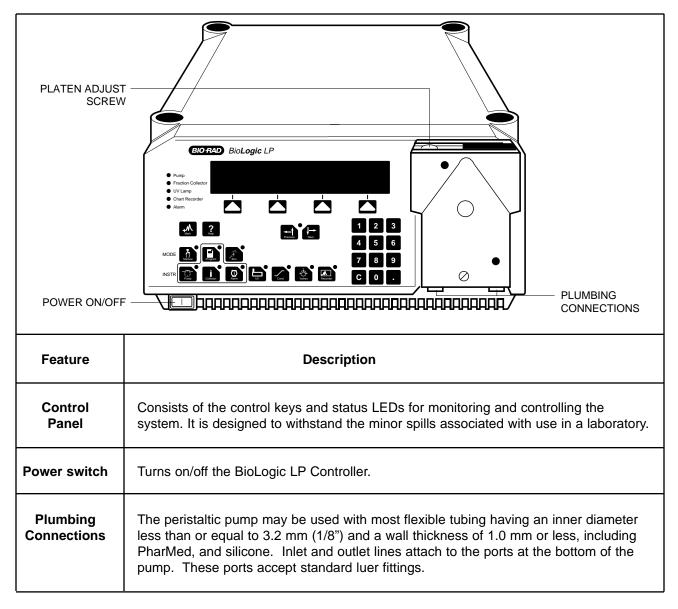
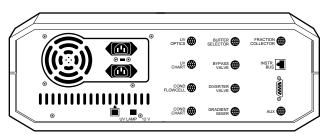


Table 3.
BioLogic LP Controller's Rear Panel Connectors



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Connector	Description
	UV Optics: For connecting the UV Optics module to the system.
	UV Chart : For UV signal output to a single or dual pen chart recorder. In addition, when the Bio-Rad Model 1327 is used, chart recorder Pen Up/Down, Stop/Start commands, and event marks are sent from this port.
	The Bio-Rad Model 1327 dual pen recorder needs an 8 pin mini-DIN to standard DIN cable (System Cable 2) available from Bio-Rad.
	Generic chart recorders require an 8 pin mini-DIN to banana plug cable (System Cable 4) available from Bio-Rad.
	The chart recorder should be set to 1V full scale.
	Cond Flowcell: For connecting the Conductivity Flow Cell to the system.
	Cond Chart: For conductivity signal output to a single or dual pen chart recorder. An 8-pin mini-DIN to banana plugs 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 1V full scale.
	Buffer Selector, Bypass, and Diverter Valves: These connectors are for connecting Bio-Rad's low pressure solenoid valves (SV-5 Buffer Select and SV-3 Bypass and Diverter valves) to the system.
	Proportioning valve/Mixer module: This is a combination gradient proportioning valve and mixer used for the forming of linear gradients as specified by the Method. The proportioning valve proportions, and then the dynamic mixer rapidly mixes the two solutions.

Table 3. (continued) BioLogic LP Controller's Rear Panel Connectors

Connector	Description	
	Fraction Collector: The BioLogic LP supports the Bio-Rad Model 2110 and Model 2128 fraction collectors, as well as other fraction collectors. This port sends "Advance" signals to the fraction collector.	
4	Instrument bus (phone jack connector): This connector is reserved for internal Bio-Rad use.	
	Test Port: This connector is reserved for internal Bio-Rad use.	
	Aux connector: This connector is reserved for internal Bio-Rad use.	
	UV Lamp: This specialized 6-pin square port provides electrical power to the mercury lamp in the UV Optics module's lamp housing.	
•	12 Volt: This is a 12 V DC power source designed to power optional equipment.	
	Power Connectors: There are two power connectors. They are keyed so that you can plug a fraction collector into one power connector and the BioLogic LP Controller's power cord into a power strip. This arrangement means the fraction collector's power switch can be left in the ON position. The BioLogic LP Controller's power ON/OFF switch then turns on/off both the BioLogic LP Controller and the fraction collector.	

2.2 PROPORTIONING VALVE/MIXER MODULE

This is a combination proportioning valve and mixer. The solenoid valve proportions the two solutions for the purpose of forming linear gradients, and the dynamic mixer rapidly mixes them. It can be mounted to the system rack or left free-standing.

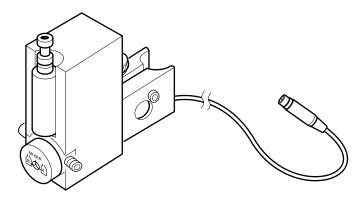


Figure 2. Proportioning valve/Mixer module

2.3 UV DETECTOR

The UV Detector, which consists of the UV Optics Module and the control circuitry within the BioLogic LP Controller, is a single beam, fixed wavelength UV absorbance detector specifically designed for protein chromatography. The UV Optics Module can be positioned close to a column outlet, minimizing system dead volume and remixing of peaks. When connecting the UV Optics Module to the system, note the flow direction arrows on the top of the UV Optics Module's case. It is important that the flow direction through the flow cell is correct.

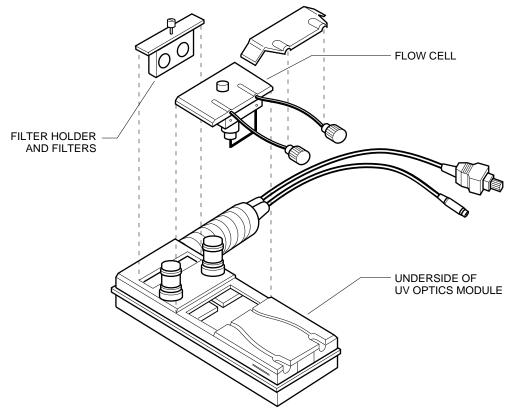


Figure 3. UV Optics Module

The AU readings from the BioLogic LP UV detector will not correspond directly to absorbance readings from a spectrophotometer. The primary reason for this is the different path lengths; light bandwidths and other factors have an effect as well. This has no effect on the UV monitor's ability to detect protein.

Eleven fixed UV absorbance ranges, from 5.0 to .001 Absorbance Units Full Scale (AUFS), are available. The user may choose a "custom" range using the "Set Max" function. Refer to Table 9.

The UV Optics Module consists of a low pressure mercury lamp, 280nm and 254nm filters, and a flow cell. The filters are both held by a single tray. The flow cell, which uses luer fittings, has a 2 mm path length. It has an internal volume of $80 \mu l$ and an illuminated volume of $3 \mu l$.

The UV Optics Module receives power from the BioLogic LP Controller, 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 BioLogic LP Controller.

Figure 3 shows how the flow cell and the filters can be changed. To remove the flow cell, you will need to remove the retaining clip from the bottom of the optics module and pull the luer connectors away from their holders. Then loosen the thumbscrews and remove the flow cell. When inserting the replacement flow cell, make sure the O-ring is securely placed on the flow cell. To change the filter, loosen the filter's thumbscrew and lift out and rotate the filter holder. The selected wavelength is indicated by the raised arrow on the bottom of the case.

WARNING: To avoid exposure to UV radiation, turn off the UV lamp when changing filters and flow cells.

2.4 CONDUCTIVITY DETECTOR

The Conductivity Detector consists of a small, portable Conductivity Flow Cell and the control circuitry which resides in the BioLogic LP Controller. This monitoring system provides a means to check equilibration conditions and the formation of a salt gradient, for example, in ion-exchange chromatography and hydrophobic interaction chromatography. This data helps in optimizing purification protocols and column cleaning procedures.

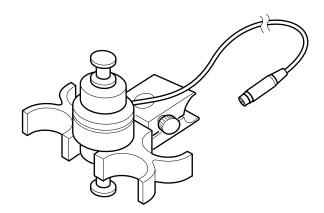


Figure 4. Conductivity Flow Cell and Separate Holder

The Conductivity Flow Cell consists of the following:

- Flow cell with Inlet/Outlet ports: For connection of the tubing, using luer fittings. The flow cell allows flow in either direction.
- Signal Cable: For connection to the back of the BioLogic LP Controller. Electrical power for the Conductivity Flow Cell is drawn off the signal cable.

The flow cell can be plumbed immediately after the UV Optics Module's flow cell or at any other point in the flowpath. A separate holder for the Conductivity Flow Cell is mounted to the system rack. The swept volume in the cell is a nominal $2\,\mu l$.

Conductivity is measured in milli-Siemens (mS). For accurate measurement, the flow cell must be calibrated. Refer to Table 10.

Nine fixed Conductivity ranges, from 500 to 0.5 mS Full Scale (mSFS), are available. The user may choose a "custom" range using the "Min/Max" function. Refer to Table 10.

2.5 BIO-RAD SYSTEM OPTIONS

Bio-Rad offers a number of system options for use with your BioLogic LP. The following instrument options are described in this section.

- Models 2110 and 2128 Fraction Collector
- Model 1327 Chart Recorder

2.5.1 Model 2110 Fraction Collector

The Model 2110 collects up to 80 fractions in a motor-driven carousel. It uses standard 13 x 100 test tubes. An optional adapter is available for use with 1.5 ml microcentrifuge test tubes.

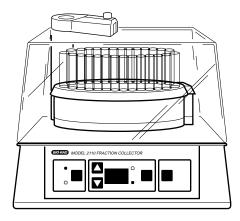


Figure 5. Model 2110 Fraction Collector (Dust Cover is optional)

The Model 2110 is connected to the BioLogic LP Controller via **System Cable 1**. When controlled by the BioLogic LP Controller, fraction collection is by time or volume. The numeric display screen on the Model 2110 is inactive while the fraction collector is under the control of the BioLogic LP. Only the fraction collector's Advance key is active during this time.

The SV-3 diverter valve is required by the Model 2110 when doing any collection scheme other than "Collect All." The SV-3 diverts flow away from the fraction collector when fractions are not being collected. Collection parameters such as Collect All, Threshold, Collection Windows, and Threshold + Collection Windows become available in Program mode.

The Model 2110 is plumbed to the BioLogic LP using Tygon tubing. The Tygon tubing is inserted directly into the fraction collector's drop former, without the need for additional fittings.

The Model 2110 fraction collector is described in detail in its separate documentation.

2.5.2 Model 2128 Fraction Collector

The Model 2128 provides X/Y motion drop-dispensing, accommodating a wide range of tube diameters and lengths, microtiter plates, microtubes, and "bottle size" fractions. It is ideally suited for both analytical and preparative applications.

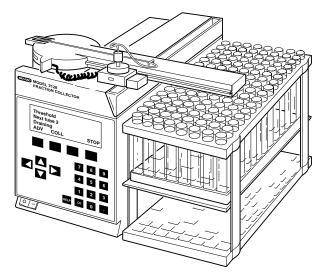


Figure 6. Model 2128 Fraction Collector

The Model 2128 is connected to the BioLogic LP Controller via the following cables:

- System Cable 15: This is required whenever the Model 2128 is to be controlled by the BioLogic LP Controller. It transmits the "Advance" commands. (Note: Only three of the 15 pins on the D-connector are used.)
- **System Cable 3**: This is required when the Model 2128's arm-mounted diverter valve is used, or when no diverter valve is used. This cable transmits the "Collect/waste" commands from the BioLogic LP, which tells the fraction collector when to move the drop head to the waste trough, or when the optional arm-mounted diverter valve is to switch between collect and waste.

When connected as described above and place in its ECONO mode, the Model 2128 is controlled by the BioLogic LP Controller. Collection and tube advance is controlled by the BioLogic LP. Programming mode supports the following collection schemes: Collect All, Threshold, Collection Windows, and Threshold + Collection Windows (all with Delay volume, if desired). The Model 2128's optional diverter valve mounts on the collector's arm and minimizes liquid spills during fraction advances.

The Model 2128 is plumbed to the BioLogic LP Controller using Tygon tubing and either of the following fittings:

- To connect directly to the drop head, use the luer adaptor (provided with the Model 2128 Fraction Collector) to adapt the Tygon tubing from the BioLogic LP to the 1/16" tubing leading to the drop head.
- To connect to the optional arm-mounted diverter valve, use the luer adaptor (provided with the Model 2128) to adapt the Tygon tubing from the BioLogic LP to the 1/16" tubing leading to the "Common" port of the diverter valve.

This instrument is described in detail in its separate documentation.

2.5.3 Model 1327 Chart Recorder

When used with the BioLogic LP, the Model 1327 Chart Recorder automatically starts when a method begins and stops when the method ends. The BioLogic LP's Manual mode operation of the chart recorder may be used to start and stop the chart recorder.

The following cables are required to connect the Model 1327 Chart Recorder to the BioLogic LP Controller:

- **System Cable 2:** Through this cable, the BioLogic LP outputs the UV analog data signal, pen up/down, and Stop/Start commands to the Model 1327 chart recorder. It connects to the BioLogic LP's **UV Chart** port and to the chart recorder's DIN connector.
- System Cable 4: Through this cable, the Conductivity analog data signal is sent from the BioLogic LP to the Model 1327 Chart Recorder. The cable connects to the BioLogic LP's Cond Chart port and to the chart recorder's channel 2 banana plug connectors.
- **Power cable and adapter**: Operating power for the Model 1327 Chart Recorder is supplied via the jack next to the chart recorder's DIN connector. (Note: The Model 1327 Chart Recorder may also be run on batteries.)

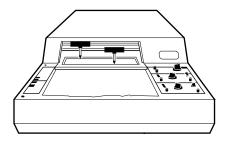


Figure 7. Model 1327 Chart Recorder

In addition, following chart recorder controls should be set:

- All chart recorder controls with a green setting should be set to their green setting.
- Paper speed dial: Select a chart speed appropriate for the separation being performed.
- Manual paper feed: Using this switch, select Paper "fast feed" to quickly advance the paper to the desired position.
- Baseline adjustment dial: These dials allow for pen position adjustment. The "zero" position of each
 pen is adjusted using the thumbwheel for each channel. When adjusting the pen's zero position,
 make sure that the BioLogic LP instrument (UV or Conductivity Monitor) is zeroed first.

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

This instrument is described in detail in its separate documentation.

2.6 NON-BIO-RAD SYSTEM OPTIONS

Non-Bio-Rad fraction collectors and chart recorders may be used with the BioLogic LP, as discussed in the following sections.

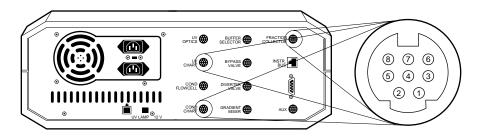


Figure 8. Pin Assignments for BioLogic LP's Fraction Collector and Chart Recorder Connectors

2.6.1 Non-Bio-Rad Fraction Collectors

A non-Bio-Rad collector may be used with the BioLogic LP, providing its tube advance function can be initiated by a TTL signal with a duration of 100 milliseconds or greater.

Use **System Cable 7** to connect a non-Bio-Rad fraction collector. Pin-outs for the BioLogic LP's Fraction Collector port are listed below and shown in Figure 8. Pin 5 is the TTL control wire, and pin 7 is the logic ground. A wire color to pin number chart is included with **System Cable 7**. See the pin-out information in your collector manual.

- **Pin 1**: The function of this pin differs, depending on your selection in Manual mode operation of the fraction collector. (See Table 7, Manual Mode Operation: Fraction Collector for details on selecting a fraction collector.) Manual mode offers the following selections:
 - **Other** or **2128**: The BioLogic LP software allows you to select "Other" when indicating a non-Bio-Rad fraction collector. (Selecting 2128 functions similar to Other.) In this mode, this pin is normally "low" (0 volts). It goes "high" (5 volts) for 100 milliseconds when a tube change is commanded.
 - **2110**: When the Model 2110 fraction collector is selected, this pin is normally "high" (5 volts). It goes "low" (0 volts) for 100 milliseconds when a tube change is commanded.
- Pins 2 4: Not connected.
- **Pin 5**: The function of this pin differs, depending on your selection in Manual mode operation of the fraction collector. (See Table 7, Manual Mode Operation: Fraction Collector for details on selecting a fraction collector.) Manual mode offers the following selections:
 - **Other** or **2128**: When Other (or Model 2128) fraction collector is selected, this pin is normally "high" (5 volts). It goes "low" (0 volts) for 100 milliseconds when a tube change is commanded.
 - **2110**: In this mode, this pin is normally "low" (0 volts). It goes "high" (5 volts) for 100 milliseconds when a tube change is commanded.
- Pin 6: Not connected.
- Pin 7, Digital Ground: This is the "ground reference" pin for all control inputs and outputs on this
 connector.
- Pin 8, Event Mark In: This pin is normally high (5 volts). When an external device such as a fraction collector pulls it "low" for 100 milliseconds or more, the BioLogic LP generates an event mark on the analog output to the Chart Recorder's channel 1.

If you are using a non-Bio-Rad fraction collector, use an SV-3 diverter valve to enable advanced features such as collection by Threshold and/or Collection Windows. If this optional valve is **not** used, then only Collect All is available.

2.6.2 Non-Bio-Rad Chart Recorders

A non-Bio-Rad chart recorder may be used with the BioLogic LP. A mini-DIN to banana plug cable (**System Cable 4**) is used to connect the BioLogic LP Controller's UV Chart port to the chart recorder. The input signal voltage should be set to 1V for both BioLogic LP UV and Conductivity signals.

The pin outs for the BioLogic LP's UV Chart port are listed below and shown in Figure 8:

- Pin 1, Stop Paper: This pin is high (5 volts) when a method is running or when the BioLogic LP's Manual mode is used to make the chart recorder run. When the method ends, or Manual mode is used to stop the chart recorder, the BioLogic LP pulls this pin "low" (0 volts) and the chart paper stops feeding.
- Pins 2 and 3: Not connected.
- **Pin 4, Integrator (+)**: This pin sends an unfiltered, unscaled signal to integrators or other data acquisitions systems. An absorbance of 2 AU will cause a 1 volt signal at this pin, regardless of UV Monitor range settings. When using this output, connect the negative lead to pin 7, Analog ground.
- **Pin 5, Chart Recorder (+):** This pin is connected to the positive lead of the chart recorder channel 1, which displays the UV absorbance trace. This signal is 0 volts when the UV Monitor reads 0 AU, and 1 volt when the UV monitor reads "full scale".
- **Pin 6, Pen Lift**: This pin is "low" (0 volts) when a method is running, or when the BioLogic LP Manual mode is used to make the chart recorder run. When the BioLogic LP allows this pin to go "high", the pens lift from paper.
- Pin 7, Chart Recorder (–), analog/digital ground: This pin is connected to the negative lead of the chart recorder channel, which displays the UV absorbance trace. It also serves as the negative lead for the integrator signal and the negative connection for all digital signals on this connector.
- Pin 8: Not connected.

The pin outs for the BioLogic LP's Cond Chart port are listed below and shown in Figure 8:

- Pins 1 4: Not connected.
- **Pin 5, Chart Recorder (+)**: This pin is connected to the positive lead of the chart recorder's channel 2, which displays the conductivity trace. This signal is 0 volts when the Conductivity monitor reads the programmed "minimum" conductivity value, and 1 volt when the conductivity monitor reads the programmed "maximum" value.
- Pin 6: Not connected.
- **Pin 7, Chart Recorder (–), analog ground**: This pin is connected to the negative lead of the chart recorder's channel 2, which displays the conductivity trace.
- Pin 8: Not connected.

3.0 SYSTEM CONNECTIONS

When setting up the system, consider the amount of bench space available, the instruments to be used with the system, and the devices to be used with the System Rack.

The BioLogic LP can be set up wherever there is available bench space and sufficient power outlets, including in 4° cold rooms or cold boxes. When used in a cold environment, it is recommended that you leave system power "ON" to minimize condensation.

All BioLogic LP systems will include as a minimum the following:

- Controller
- System Rack (single tray configuration)
- UV Optics Module
- Conductivity Flow Cell

- Proportioning valve/Mixer module
- MV-6 Inject Valve
- Fittings kit
- Tubing

Your BioLogic LP system may include components or instruments not listed above. Refer to the separate supporting documentation for each of the following types of devices:

- Bio-Rad Model 2128 or Model 2110 Fraction Collector
- Bio-Rad Model 1327 Chart Recorder
- additional valves
- instruments not purchased from Bio-Rad, such as a generic fraction collector or chart recorder

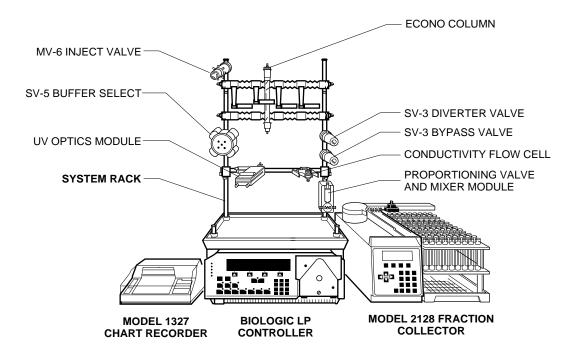


Figure 9. Example of a BioLogic LP System

To set up the BioLogic LP System:

1. BioLogic LP Controller.

- a. Place the BioLogic LP Controller on the bench and turn the unit so that the back side faces you.
- b. Connect the power cable. Do not turn on the unit.

2. System Rack.

Assemble the Rack and place it on the BioLogic LP Controller, as described below.

- a. 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:
 - The long rods have several grooves. Attach the collars to the groove closest to the end of the rod. Attach the collars so that the taper is toward the end of the rod.
 - The short rods each have only one groove. Fit the collars onto the grooves so that the collars flare out toward the farthest end of the rod. Refer to Figure 10.
- b. Insert the two long rods into the holes at the back of the tray, as shown in Figure 10.

Note: The rods are inserted from underneath the tray such that they produce a firm fit in the holes.

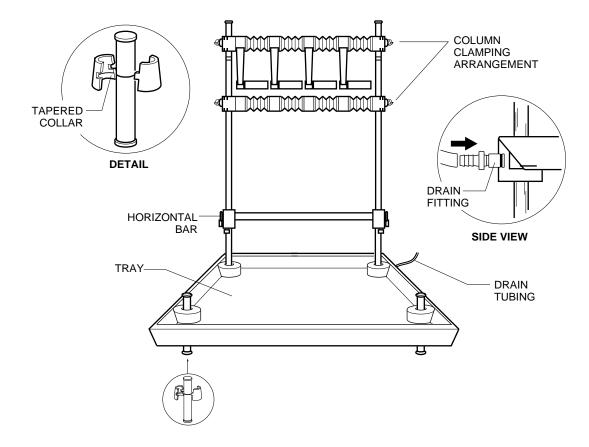


Figure 10. Rack Assembly

- c. Insert the two short rods into the remaining holes.
- d. Mount the 2-piece column clamping arrangement across the two long rods using the attached thumbscrews.
- e. The horizontal bar may be positioned between the upright bars using the rod clamps (tightened by the Allen wrench) supplied with the system.
- f. Place the rack into the four corner holes of the BioLogic LP Controller. Since the back of the BioLogic LP Controller is facing you, you will want the back of the rack (the side with the two long rods) also facing you.
 - If a hole is covered by a green cap, remove the cap.

3. Proportioning valve/Mixer module.

- a. Attach the Proportioning valve/Mixer module to a suitable position on the vertical bar using the module's rod clamp.
- b. Connect the signal cable from the Proportioning valve/Mixer module to the port marked "Gradient Mixer" on the rear of the BioLogic LP Controller.

4. Buffer Select valve.

- a. If you will be using the SV-5 Buffer Select valve, attach the valve to a suitable position on the horizontal bar using the valve's rod clamp.
- b. Connect the signal cable from the Buffer Select valve to the port marked "**Buffer Selector**" on the rear of the BioLogic LP Controller.

5. UV Optics Module and Conductivity Flow Cell.

- a. Attach the UV Optics Module to a suitable position on the horizontal bar using a rod clamp to hold one of the module's two foot-posts.
- b. Connect the power cable (square connector) from the UV Optics Module lamp into the port marked "UV Lamp" on the rear of the BioLogic LP Controller.
- c. Connect the UV Optics Module's signal cable (mini-DIN connector) to the port marked "**UV Optics**" on the rear of the BioLogic LP Controller.
- d. Connect the Conductivity Flow Cell's combined power and signal cable (mini-DIN connector) to the port marked "Cond. Flowcell" on the rear of the BioLogic LP Controller.
- e. Attach the Conductivity Flow Cell's holder to a suitable position on the horizontal bar using the holder's rod clamp.
- f. The Conductivity Flow Cell is designed to be held in its holder, but may be placed anywhere in the fluid path as desired by the user.

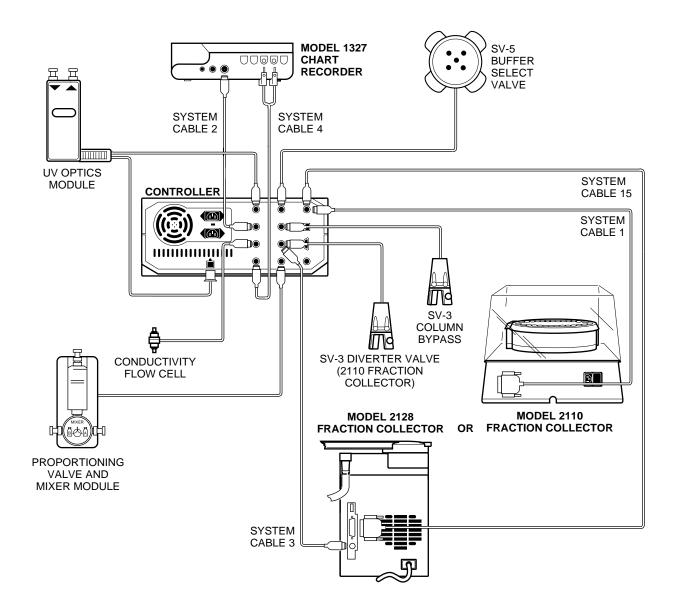


Figure 11. System Cable Connections

6. Chart Recorder; Model 1327.

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

- a. Connect System Cable 2 between the BioLogic LP Controller and the recorder as follows:
 - The mini-DIN connector is connected to the port marked "UV Chart" on the rear of the BioLogic LP Controller.
 - The DIN connector is connected to the single DIN port 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.

- b. Conductivity signals are recorded on channel 2 of the recorder using **System Cable 4** as follows:
 - The mini-DIN connector is connected to the port marked "Cond. Chart" on the rear of the BioLogic LP Controller.
 - The banana plugs are connected to the ports marked **CH 2** on the side of the recorder (red wire to +, black wire to ground ⊥).
- c. Set both channel inputs on the recorder to 1V. Set all other switches to their position marked in green. Connect the power adapter to a wall outlet, and plug the power lead into the chart recorder.

7. Fraction Collector; Model 2110.

- a. The Model 2110 fraction collector may be positioned on the rack shelf or on the bench.
- b. Connect **System Cable 1** between the BioLogic LP Controller's Fraction Collector port and the fraction collector as shown in Figure 11 and described below:
 - The 9-pin D connector is connected into the single D port on the rear of the Model 2110.
 - Connect the power cable. (A power cable is available to connect the fraction collector to the BioLogic LP Controller, eliminating the need for a wall outlet.)
- c. Position the SV-3 valve on the rack using the integral clamp.
- d. Connect the SV-3 valve cable mini-DIN connector to the **Diverter Valve** port.

8. Fraction Collector; Model 2128.

- a. Place the Model 2128 fraction collector on the bench, next to the BioLogic LP.
- b. Connect **System Cable 15** between the BioLogic LP Controller's Fraction Collector port and the fraction collector as shown in Figure 11. The 15-pin D connector is connected into the single D port on the rear of the Model 2128.
- c. Connect System Cable 3 between the BioLogic LP Controller's Diverter Valve port and the fraction collector as shown in Figure 11. The mini-DIN connector is connected into the single mini-DIN port on the rear of the Model 2128.
- d Connect the power cable. (A power cable is available to connect the fraction collector to the BioLogic LP Controller, eliminating the need for a wall outlet.)

When all the connections have been made, turn the units around. Plug all the power cables into an approved power-strip. The system can now be plumbed.

4.0 SYSTEM PLUMBING

This chapter begins with discussion of plumbing practices and general guidelines, followed by a general procedure for plumbing the major system components. Later sections discuss how to plumb Bio-Rad's low pressure valves.

4.1 GENERAL GUIDELINES FOR PLUMBING THE SYSTEM

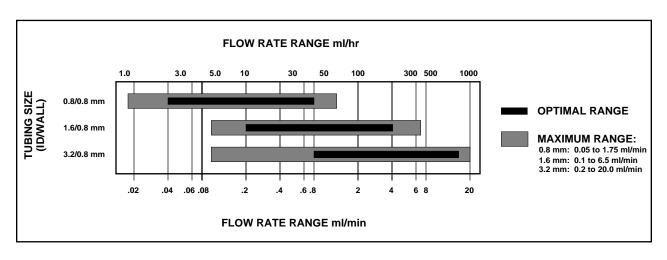
The BioLogic LP uses the following tubing:

- System tubing: 0.8mm ID or 1.6mm ID Tygon tubing.
- Pump Tubing: 0.8mm ID, 1.6mm ID, or 3.2mm ID PharMed tubing. Refer to the following table when selecting a tubing.

Warning

Do not use pump tubing with wall thickness greater than 1.0 mm. Using tubing with a wall thickness greater than 1.0 mm can damage the pump and void the warranty.

Table 4
Comparison of Flow Rate Ranges for Different Tubing IDs



All plumbing connections require Luer fittings. A fittings kit is included with the BioLogic LP system. The figure below shows the different Luer connectors available for use in plumbing the BioLogic LP system.

 MALE LUER FOR SYSTEM TUBING CONNECTIONS		FEMALE LUER FOR PUMP TUBING
MALE-TO-MALE CONNECTOR	T	FEMALE 'T' CONNECTOR

Figure 12. Luer Fittings

When plumbing the system, be sure to keep tubing lengths to a minimum. Also, all fittings should be fingertight.

The procedures for plumbing the pump and all components in the BioLogic LP system are provided on the following pages.

4.2 ADJUSTING THE PLATEN AND PLUMBING THE PERISTALTIC PUMP

The peristaltic pump requires the following tubing lengths:

- PharMed 179 ±1.5mm
- Silicone 171±1.5mm

Note: PharMed is preferred. (Tygon should not be used in the pump head because it fatigues rapidly.) If you are not using pre-cut tubing, cut the tubing to the lengths indicated above.

 Proper adjustment of platen pressure increases flow stability, minimizes flow pulsation, and prolongs the life of the tubing. To adjust platen pressure, use the platen adjustment screw. First turn the platen adjust screw counterclockwise until it stops. Then turn the screw clockwise according to the table below:

	Number of Turns
Tubing ID	From Fully Open
0.8 mm (1/32")	5
1.6 mm (1/16")	4
3.2 mm (1/8")	3

Note: Overtightening the platen reduces flow rate and shortens tubing life. A loose platen will cause the flow rate to decrease as the backpressure increases.

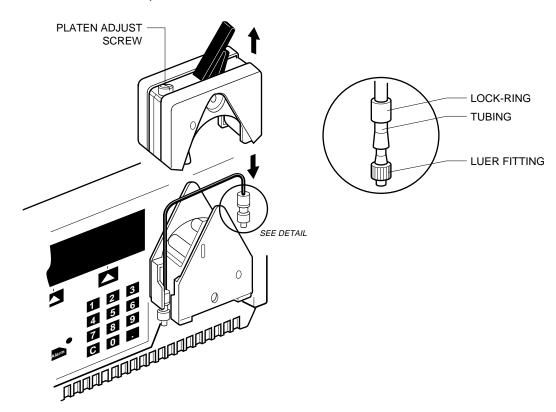


Figure 13. Plumbing the BioLogic LP Controller's Pump

- 2. Pull the platen cam lever away from the pump head to unlock the platen and slide the platen away from the pump head frame assembly, exposing the rollers.
- 3. Slip a lock-ring onto one end of the tubing. The lock rings must be put on before luer fittings, and the recessed parts of the lock rings should face each other. The size and color of each lock ring is as follows:

<u>Tubing ID</u>	Lock Ring Color
0.8 mm (1/32")	Red
1.6 mm (1/16")	Orange
3.2 mm (1/8")	Yellow

Insert a barbed female luer-fitting into the same end until the tubing reaches the flange of the fitting. Clamp the luer fitting into place by sliding the lock-ring along the tubing over the barbed fitting. Repeat the procedure for the other end of the tubing. For flow rates greater than 20.0 ml/min see the note below.

- 4. Insert one end's fitting into one of the tubing retaining brackets of the pump head. Pull the tubing around the rollers, and insert the other end's fitting into the bracket on the opposite side of the pump head. Use the same bracket on each side of the pumps, either the front brackets or the back brackets.
- 5. Slide the platen back into the pump head frame assembly until it contacts the tubing. Press the cam lever in toward the pump head, locking the platen up against the tubing and rollers. Note that the platen can be inserted with the cam lever on the left or the right.

Note: To obtain flow rates from 20.0 to 40.0 ml/min prepare two pieces of 3.2 mm pump tubing as in step 3. Use two Female "T" Connectors, see figure 12, and four short lengths of tubing with male luer fittings on both ends to join the two pieces together. Place both pieces of pump tubing into the pump head as in step 4. For accurate flow rates when this is done you must perform User Calibration. Refer to section 6.2.2.



Caution

Each time the pump's tubing is changed, especially if the tubing size is changed, the pump must be recalibrated. Refer to Section 6.2, Pump Calibration.

4.3 PLUMBING THE SYSTEM

1. Plumbing the inlets to the Proportioning valve/Mixer module.

- a. Cut two suitable lengths of Tygon tubing (0.8mm ID or 1.6mm ID).
- b. Attach the barbed end of a male luer to one end of each piece of tubing. (Warming the Tygon tubing in warm water makes it more flexible and easier to work with.)
- c. Connect each fitting to one inlet port of the Proportioning valve. Twist the fitting clockwise to lock. Do not over-tighten.
- d. Immerse the ends of the tubing in a container of high quality water or buffer.

2. Plumbing the outlet from the Proportioning valve/Mixer module.

If you are **not** using the optional SV-5 Buffer Select valve, plumb the Mixer module's outlet to the left port of the pump. Then proceed to step 5.

If you are plumbing to the optional Buffer Select Valve:

- a. Cut a suitable length of Tygon tubing that will reach from the Proportioning valve/Mixer module outlet to the SV-5 Buffer Select valve.
- b. Attach male luer fittings to each end.
- c. Connect the tubing to the outlet port on the Proportioning valve/Mixer module and to port A/B of the SV-5 Buffer Select valve. Twist clockwise to lock. Do not over-tighten.

3. Plumbing the outlet from the SV-5 Buffer Select Valve to the BioLogic LP Controller's pump.

- a. Cut a length of Tygon tubing that will reach from the SV-5 Buffer Select valve to the BioLogic LP Controller's pump.
- b. Attach male luer fittings to each end.
- c. Connect the fitting to the Common port on the SV-5 Buffer Select valve and to the left port of the pump. Twist clockwise to lock. Do not over-tighten.

4. Plumbing from Buffer Reservoirs C through E to the Buffer Select Valve.

- a. Cut a length of Tygon tubing that will reach from each Buffer Reservoir to the SV-5 Buffer Select valve
- b. Attach the male luer to one end of each piece of tubing.
- c. Screw the tubing into inlet ports C through E of the SV-5 Buffer Select valve. Twist clockwise to lock. Do not over-tighten.
- d. Immerse the ends of the tubing in a container of high quality water or buffer.

5. Plumbing from the BioLogic LP Controller's pump to the MV-6 Inject Valve.

- a. Use the screw fitting on the MV-6 Inject valve to secure the valve to the system rack. Turn the MV-6 knob counterclockwise so it matches that shown in Figure 14.
- b. Cut a suitable length of Tygon tubing that will reach from the BioLogic LP Controller's pump to the MV-6 Inject valve.
- c. Attach male luer fittings to each end.
- d. Connect the tubing to the pump's right port and to the MV-6 Inject valve and secure firmly using a twisting action. Turn clockwise to lock; do not overtighten.

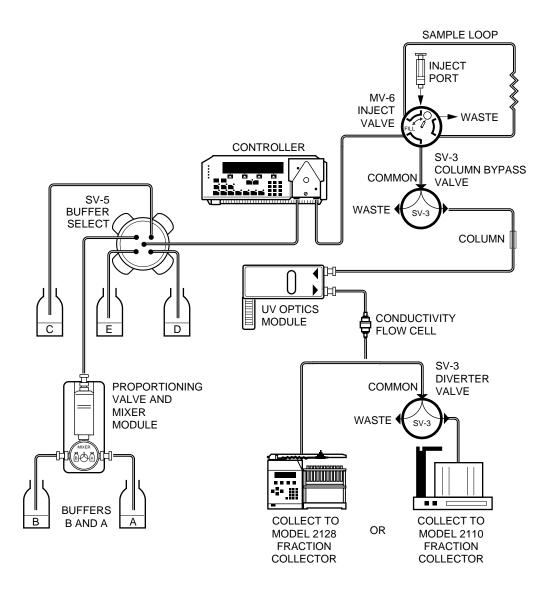


Figure 14. System Plumbing

6. Plumbing the MV-6 Valve's Sample Loop.

Plumb the inject valve's sample loop according to Figure 14. Use male luer fittings at each end of the loop tubing. A sample loop of any volume can be prepared. Volumes may be determined empirically, or by following the example below:

Total volume of sample = Valve volume + Fittings volume + Tubing volume where,

Volume valve passage and fittings = .325 ml

Tubing volume = Length of tubing x Volume/cm, as determined from the following:

ID	Volume/cm
0.8	.005 ml/cm
1.6	.020 ml/cm
3.2	.08 ml/cm

Example: A 2 ml sample loop using 1.6 mm ID tubing:

a. Subtract volume of fittings and valve passage:

2 ml - 0.325 ml = 1.675 ml

b. Divide the tubing volume needed by the volume/cm of tubing:

 $1.675 \text{ ml} \div 0.020 \text{ ml/cm} = 83.75 \text{ cm of tubing}$

In this example, the volume of the valve and fittings, plus the internal volume of 83.75 cm of the 1.6 mm ID tubing, equals 2 ml.

7. Plumbing the SV-3 Bypass valve. (Optional)

Note: If you will not be using the optional SV-3 Bypass valve, proceed to the next step.

- a. Connect a length of Tygon tubing between the MV-6 Inject valve and the SV-3 Bypass valve common port (labeled "**Common**") using male luer fittings.
- b. Connect a piece of Tygon tubing from the 'Bypass" port of the SV-3 Bypass valve (see figure 14) to a suitable waste container.

8. Plumbing the Column.

Note: The column will be positioned in the fluid path *after* the system is purged of air. In its place, temporarily connect the column inlet line to the column outlet line.

- a. Plumb the column inlet as follows:
 - If you are using an SV-3 Bypass valve, cut a length Tygon tubing that will reach between the SV-3 Bypass valve and the column inlet. Attach male luer fittings to each end. Connect one end to the SV-3 valve's "Collect/Column" port.
 - If you are **not** using the SV-3 Bypass valve, cut a length that will reach between the MV-6 Inject valve and the column inlet. Attach male luer fittings to each end. Connect one end to the MV-6 valve's "Outlet" port.
- b. To plumb the column outlet, cut a length of tygon tubing that will reach from the column outlet to the UV Optics Module. Attach a male luer fitting at one end and a female luer fitting at the other.
- c. Connect the column inlet line to the column outlet line. This will be disconnected to insert the column after purging the system.

9. Plumbing the UV Optics Module and Conductivity Flow Cells.

a. Connect the column outlet line to the inlet port of the UV Optics Module.

(Arrows on the UV Optics Module indicate flow direction.)

- b. Cut a short length of Tygon tubing and attach luer fittings to each end.
 Screw one end into the output port of the UV Optics Module's flow-cell and the other end into the Conductivity Flow Cell. (Note: The Conductivity Flow Cell is bi-directional.)
- c. Place the Conductivity flow cell into its holder.

10. Connection to a Model 2110 Fraction Collector or Non-Bio-Rad Fraction Collector. Note: This step involves the use of an SV-3 Diverter valve.

- a. Connect a piece of Tygon tubing between the Conductivity flow cell and the SV-3 Diverter valve common port (labeled "Common") using the luer fittings.
- b. Connect a piece of Tygon tubing from the SV-3 Diverter valve's "Waste" port for use as a waste line.
- c. Connect a piece of Tygon tubing from the SV-3 Diverter valve's "Collect" port to the Model 2110 fraction collector drop-head. This tubing sits inside the drop-head, and no fittings are required.

11. Connection to a Model 2128 Fraction Collector.

For further discussion on plumbing the Model 2128, refer to the Model 2128 Fraction Collector Instruction Manual.

Note: The tubing's length must allow unrestricted movement of the fraction collector arm.

When using the Model 2128 Fraction Collector with the BioLogic LP, you have three options for diverting "waste" fluid.

- a. No diverter valve used: In this configuration, tubing from the Conductivity flow cell is connected directly to the fraction collector's drop head. This requires a female luer adapter (supplied with the Model 2128) to connect the tubing from the Conductivity flow cell to the 1/16" tubing from the Model 2128. This will require using a barb-to-male fitting from the BioLogic LP fittings kit. (Note: Be sure that System Cable 3 is connected between the Model 2128 and the Controller's Diverter Valve port.) When you turn on the Model 2128, select "Econo" mode from the Model 2128's startup screen. The system prompts with the message "Is 2128 valve cable connected." (This refers to System Cable 3.) Answer YES.
- b. **Using the Model 2128's arm-mounted diverter valve**: Tubing from the Conductivity flow cell is connected to the diverter valve's Common port. This requires a female luer adapter (supplied with the Model 2128) to connect the tubing from the Conductivity flow cell to the 1/16" tubing from the Model 2128. This will require using a barb-to-male fitting from the BioLogic LP fittings kit.

Plumb the diverter valve's Collect port to the drop head and its Waste port to a suitable waste container. Use the 1/16" tubing and ferrules supplied with the Model 2128.

(Note: Be sure that System Cable 3 is connected between the Model 2128 and the Controller's Diverter Valve port.) When you turn on the Model 2128, select "Econo" mode from the Model 2128's startup screen. The system prompts with the message "Is 2128 valve cable connected." (This refers to System Cable 3.) Answer YES.

c. Using an SV-3 Diverter valve: Mount the valve on the System Rack so that it is as close as possible to the Model 2128 Fraction Collector. Plumb the system as shown in Figure 14. (Note: This configuration requires the valve be connected to the Diverter Valve port on the BioLogic LP.) When you turn on the Model 2128, select "Econo" mode from the Model 2128's startup screen. The system prompts with the message "Is 2128 valve cable connected." (This refers to System Cable 3.) Answer NO.

Note: If you answer YES, no fractions will be collected because there will be no System Cable 3 to send commands from the BioLogic LP. To correct the problem, you will need to use the fraction collector's control panel to "park" the drop head and arm and then de-select "Econo" mode. Then reselect "Econo" mode and answer NO to the message "Is 2128 valve cable connected."

4.4 PURGING THE SYSTEM

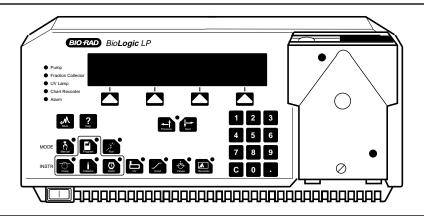
Before a method can be run, you must take proper steps to evacuate all air from the system.

- 1. Make sure the column is not connected to the system and the column inlet tubing is connected to the column outlet tubing.
- 2. Verify that all buffer inlet lines are submerged in a container of high quality water or buffer.
- 3. Turn on the BioLogic LP Controller. When you turn on the BioLogic LP Controller, the system automatically shows Manual mode for Pump operation. Verify that the arrow on the left side of line 2 of the LCD points to the right.*
- 4. Press the **Purge** softkey to run the pump at its maximum flow rate. Water or buffer will now flow through the system.
- 5. While the pump is running, press the **Buffer** softkey and cycle through all of the ports on the SV-5 Buffer Select valve. After all buffer lines have been filled with liquid, stop the pump.
- 6. Using the system's manual mode operation of the pump, set a flow rate that is safe for the column. To do this.
 - a. From the front panel of the BioLogic LP, press the Manual key, followed by the Pump key.
 - b. Using the softkeys below the front panel's display, press the **Flow** softkey.
 - c. Using the front panel's keypad, enter a flow rate that is safe for the column.
- 7. Disconnect the column inlet and column outlet lines and insert the column. Avoid introducing air to the inlet side of the column.
- 8. Press the front panel's **Run** key to purge air which may have entered the system when the column was connected.
 - *This arrow indicates the direction of flow. If the arrow points to the left press the **Flow** softkey followed by the **Forward** softkey to change the direction of flow. Finally press the **OK** softkey to confirm the change.

5.0 SYSTEM OPERATION

5.1 FRONT PANEL CONTROLS

Table 5. Front Panel Controls



Key	Description
	Softkeys : These four keys are located directly below the LCD display. They are collectively referred to as softkeys, because the function each key executes depends on the corresponding text displayed directly above the key.
Previous Next	Previous and Next keys: Use these keys to move the cursor around the screen display. Their LED is lit when these keys are active.
0 то 9	Keypad keys: 1 - 9: Use these keys to enter a numeric value or name a method.
	C: To return a field's value to zero or its default value. Decimal key: To enter a decimal point. When naming a method, this key enters a space.

Table 5. (continued) Front Panel Controls

Key	Description
Mark	Mark key: This key signals the chart recorder to put an event mark on the UV trace.
? Help	Help key : Pressing this key at any time displays information about the screen that is currently displayed. Use the Prev and Next keys to scroll through the Help screens. To exit help, again press the Help key.
PumpFraction CollectorUV LampChart RecorderAlarm	LEDs : The LEDs at the top left of the front panel are lit when the indicated function is activated. The Pump's LED flashes when the pump is running at purge speed; the UV's LED flashes when the lamp is warming up.
Modes	Mode keys : These keys allow you to select a mode of operation. The LED next to the key indicates the mode selected. For Program mode, a flashing LED indicates the mode was changed before the operation was completed. For Run mode, a flashing LED indicates the system is ready to start or is paused.
Manual	 Manual: Allows you to individually control instruments and functions. Select the instrument to be controlled using any of the keys listed in the INSTR (Instrument) line of the front panel.
Program	 Program: Allows you to write, edit, store, and recall a separation method. You can program and store up to 50 methods. Program mode's LED flashes when you select Manual mode before completing the Method.
, Run	Run: Allows you to run the currently selected method. Run mode's LED flashes when you select Pause during the run.

Table 5. (continued) Front Panel Controls

Key	Description
Instr	Instr (Instrument) keys: These keys allow you to select individual instruments to control. The selected component is indicated by its corresponding LED on the front panel and its corresponding control screen is displayed. Note that the Pump, Collector, and Alarm keys are grouped in a shaded area, indicating that these functions (pump operation, fraction collection, and alarms) are programmed as part of a separation method. Valve Buffer Select and Divert functions are programmed in a method, but Bypass functions are only selectable in Manual mode. All other functions are <i>not</i> programmed as part of a method, but may be selected prior to and during a run.
	Pump: Allows you to set the flow rate and buffer mixture for the BioLogic LP pump. Pump operating conditions are programmed as part of a method.
Collector	Collector: Allows you to set the operating conditions for the Fraction Collector. Fraction collection is programmed as part of a method.
Alarm	Alarm: Allows you to set the timer for the Alarm. Alarm values are programmed as part of a Method.
	 UV: Allows you to set the operating conditions for the UV monitor. UV settings may not be programmed as part of a method, but may be changed during a run.
Cond	Cond: Allows you to set the operating conditions for the BioLogic LP Conductivity Monitor. Conductivity settings may not be programmed as part of a method, but may be changed during a run.
Valves	 Valves: Allows you to set the operating conditions for the BioLogic LP Valves. The SV-3 Diverter valve and SV-5 Buffer Select valve functions are controlled by the method program; the SV-3 Bypass function is not programmed as part of a method.
Recorder	Recorder: Allows you to start/stop the Bio-Rad Model 1327 Chart Recorder. When a method is run, the chart recorder automatically starts when the method begins and stops (pens lift up) when the method ends. Pressing the Recorder key on the front panel during a run can start/stop chart recorder activity.

5.2 MANUAL MODE

There are three modes of operation: Manual mode, Program mode, and Run mode. Manual mode is used to prepare the system to run a programmed method, or to perform tasks that do not require a programmed method, such as simple pumping tasks. When you first turn on the system, the Manual mode's Pump Control screen is displayed so that you can purge the system, calibrate the pump, and select the buffers for your application. To select other Manual mode functions, use the Instrument keys on the front panel. The top line of each screen in Manual mode displays the following:

- Method name: The name of the currently loaded method. Changing the method name is done in Program mode.
- Mode of operation: The mode is also indicated by the key's LED being lit.

5.2.1 Manual Mode Operation of the Pump

Table 6 discusses each of the functions available in Manual mode operation of the pump. An overview of Manual mode operation of the Pump is shown in Figure 15.

Table 6.
Manual Mode Operation: Pump

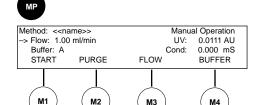
Reference Description





To begin Manual mode operation of the pump:

- a. From the Mode keys select Manual.
- b. From the Instrument keys select Pump.
- c. The main menu for manual mode operation of the pump is shown below.



Main Menu screen: The display shows the following:

- Flow: The current flow rate set for the pump. The arrow shows the direction of flow.
- UV: The current UV reading.
- Buffer A through E or %B: The currently selected buffer or a mixture, as specified by the amount of buffer B.
- · Cond: The current Conductivity reading.

Description of the soft key choices follows.

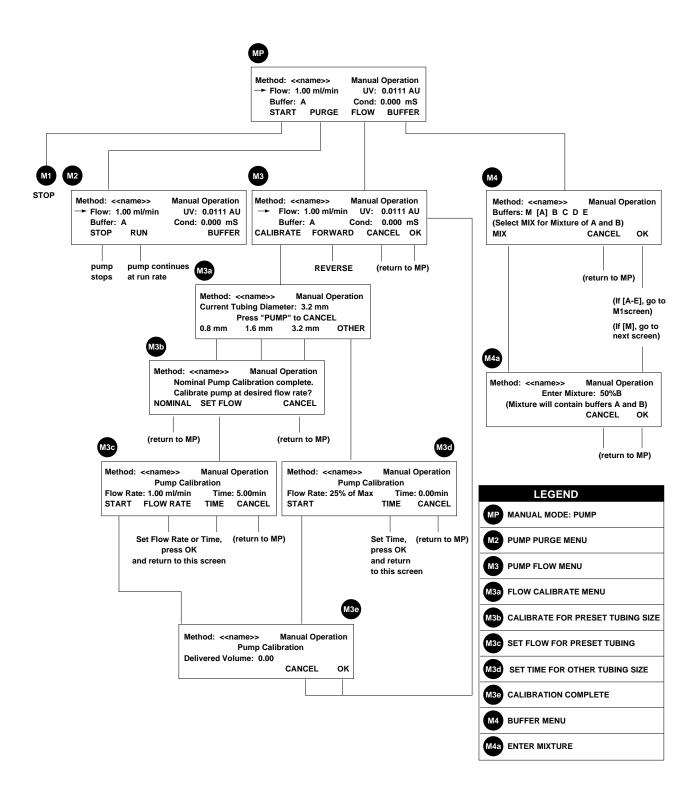


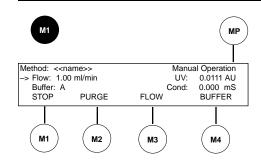
Figure 15. Manual Operation of the Pump

(Screen displays are keyed to the discussion in Table 6, Manual Mode Operation : Pump)

Table 6. (continued) Manual Mode Operation: Pump

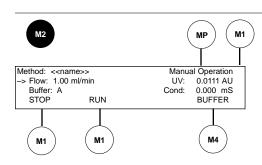
Reference

Description



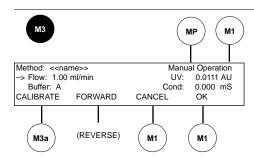
Start/Stop Screen: The first softkey in the Main Menu screen allows you to start/stop the pump using its currently set flow rate. When the pump is running, its status light is ON.

- Pressing Stop stops the pump.
- Pressing Purge increases pump speed to maximum. Refer to the Purge screen.
- Pressing Flow allows you to set the flow rate. Refer to the Flow screen.
- Pressing Buffer allows you to specify the buffer. Refer to the Buffer screen



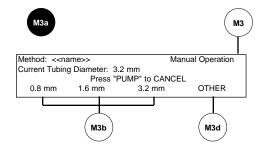
Purge Screen: Selecting **Purge** from the Main Menu screen sets the flow rate to maximum and runs the pump. The Purge screen is shown at left. During purge, the pump's status LED flashes. You then have the following choices:

- Stop: Stops the pump.
- Run: Returns to the normal set flow rate and continues operation. The status LED changes to ON.
- Buffer: Allows you to change the buffer. Refer to the Buffer screen.



Flow Screen: To change the flow rate, use the keypad to enter the desired flow rate and then press **OK**. To change the direction of the flow, press the **Forward** softkey. Forward is clockwise, and Reverse is counter-clockwise. **Note**: The arrow in the display indicates the direction of flow.

In addition, the Flow screen's menu allows you to calibrate the pump, as described below. The system uses the flow rate to determine fraction size and for gradient control when measuring by volume rather than time. The system calculates the flow rate by multiplying the pump head speed (rpm) by a flow rate factor which is determined by calibrating the pump, as discussed below. **Note**: You must select a tubing size or calibrate before running a gradient method.

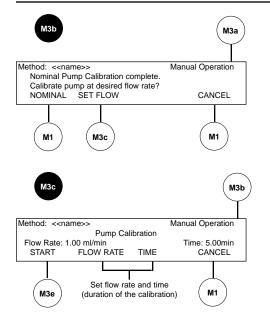


Calibrate: Values from the calibration are used by the system to determine flow rate. Calibration is based on the tubing size installed in the pump head. Calibration begins with the selection of a standard tubing size or non-standard (Other) tubing sizes.

Note: Before calibrating, check the adjustment of the platen pressure screw on the pump. Refer to section 4.2.

Table 6. (continued) Manual Mode Operation: Pump

Reference Description

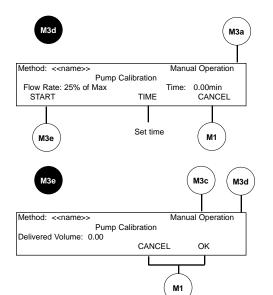


Calibration for standard tubing sizes (0.8 mm, 1.6 mm, 3.2 mm): If a standard tubing size is installed in the pump head, you may select **Nominal** to use a nominal flow rate factor. Selecting **Set Flow** allows you to do a "user calibration," which provides greater accuracy, as described below.

User Calibration (Set Flow) for improved flow accuracy:

This screen allows you to calibrate for standard tubing sizes by experimentally determining the flow rate factor. This procedure is advisable when using columns with high counter-pressure or when improved flow rate accuracy is desired. Calibration should be done with all components connected and the column installed. To calibrate:

- Once the system is set up, select **Purge** to purge air from the system. Select **Stop** to stop the pump, and place the waste tube in a graduated cylinder.
- 2. Select Flow followed by Calibrate.
- 3. Select a standard tubing size, enter Flow Rate and Time, and then press **Start** to calibrate. If more than one flow rate is to be used, calibrate for the flow rate to be used during the most critical period of the separation. For best results, set a Time of at least 5 minutes. During calibration, you can select **Stop** to shorten the calibration run.
- 4. At the end of the calibration period, when the pump has stopped, enter the total volume delivered and press OK.

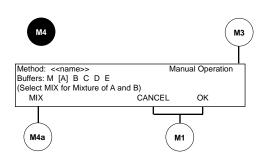


User Calibration for non-standard tubing size: To calibrate for non-standard tubing (Other), follow the procedure above. Calibration will proceed for the duration that you specify, at a flow rate of 25% of full speed. Press **Start** to begin calibration.

Calibration complete: When the calibration run is complete, enter the delivered volume and press **OK**. The pump is now calibrated.

Table 6.
Manual Mode Operation: Pump

Reference Description



Buffer screen: Selecting Buffer from the Main Menu screen displays the Buffer screen, shown to the left. This screen allows you to select the buffer to be delivered to the column. Use the **Prev** and **Next** keys to select a single buffer (A through E) or a mixture of buffers A and B. To set a mixture, select the **Mix** soft key and enter the percent of buffer B. Note: If a valve is not connected on the BioLogic LP, its letter or letters appear in lower case and cannot be selected.

5.2.2 Manual Mode Operation of the Fraction Collector

Table 7 discusses each of the functions available in Manual mode operation of the fraction collector. An overview of the Fraction Collector's Manual mode operation is shown in Figure 16.

Table 7.

Manual Mode Operation: Fraction Collector

Reference Description

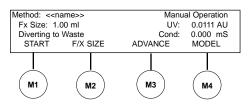




To begin Manual mode operation of the fraction collector:

- a. From the Mode keys select Manual.
- b. From the Instrument keys select **Collector**.
- c. The main menu for manual mode operation of the fraction collector is shown below.





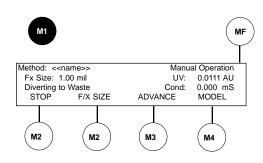
The display shows the following:

- Fraction Size: The fraction size can be specified as a volume (ml) or time (minutes).
- UV: The current UV reading.
- Diverting to Waste/ Collect: Indicates the position of the SV-3 Diverter valve.
- Cond: The current Conductivity reading.

Description of the soft key choices follows.

Table 7. (continued) Manual Mode Operation: Fraction Collector

Reference Description



Start/Stop screen: This first softkey in the Main Menu screen allows you to start/stop the fraction collector.

- Pressing Stop stops the fraction collector.
- Pressing FX Size allows you to change the fraction size.
- Pressing Advance causes the fraction collector to advance to the next tube.
- Pressing Model allows you to specify the type of Fraction Collector being used.

When the fraction collector is running, its status light is ON.

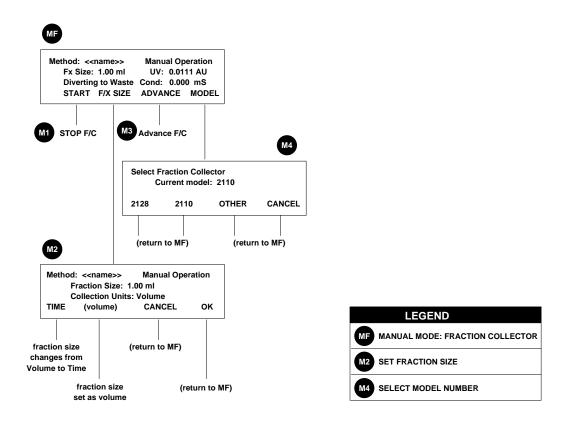
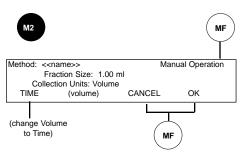


Figure 16. Manual Operation of the Fraction Collector

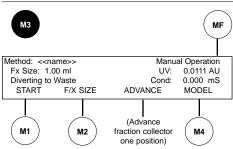
(Screen displays are keyed to the discussion in Table 7, Manual Mode Operation : Fraction Collector)

Table 7. (continued) Manual Mode Operation: Fraction Collector

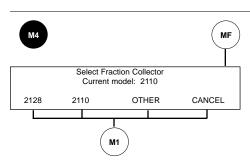
Reference Description



F/X Size screen: This allows you to set the fraction size based on either Time or Volume. If fraction size is set as a Volume, the fraction size is specified in ml and the (volume) softkey is displayed. To set a fraction size based on time, press the Time softkey.



Advance: Advances the fraction collector to the next tube.



Model screen: Select 2110 or 2128 if you are using Bio-Rad's Model 2110 or 2128 Fraction Collectors. Select **Other** for all other fraction collectors.

5.2.3 Manual Mode Operation of the Alarms

Table 8 discusses Alarm's Manual mode functions. Figure 17 shows an overview of these functions.

Table 8.

Manual Mode Operation: Alarms

Reference Description To set an alarm or turn off an alarm setting: a. From the Mode keys select Manual. From the Instrument keys select **Alarm**. The main menu is shown below. The display shows the following: Alarm Timer: This is the time at which the alarm is set to Method: <<name>>
Alarm Timer: 0.00 min Manual Operation sound and stop instruments. Stop Instruments: NO Stop Instruments: In addition to sounding an audible alarm, SET ALARM (start) (stop) CLEAR the alarm function can shut off all active instruments. (to start/pause (clears the Description of the soft key choices follows. the timer) M1 timer setting) **Start/Stop**: These are selectable when the Alarm Timer is set. Use these to start/stop timer countdown. MA Set Alarm: You can set an audible alarm to sound at any time during system operation. By pressing the **Stop On** soft key Method: <<name>> Manual Operation Alarm Timer: 0.00 min (which changes the display from "Stop Instruments: No" to Stop Instruments: NO STOP ON CANCEL (stop off) OK "Stop Instruments: Yes"), you can stop system operation when the alarm sounds. To silence the Alarm, press any key. MA Clear: Clears the Alarm Timer setting.

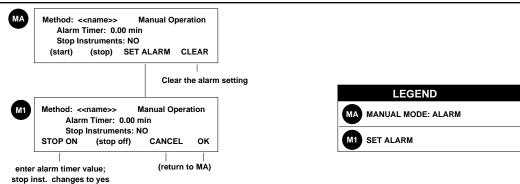


Figure 17. Manual Operation of the Alarms

(Screen displays are keyed to the discussion in Table 8, Manual Mode Operation : Alarms)

5.2.4 Manual Mode Operation of the UV Monitor

Table 9 discusses each of the functions available in Manual mode operation of the UV Monitor. For an overview of these functions, refer to Figure 18.

Table 9.

Manual Mode Operation: UV Monitor

Reference Description

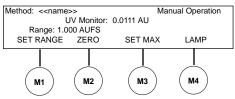




- a. From the Mode keys select Manual.
- b. From the Instrument keys select UV.
- c. The main menu is shown below.

To manually control the UV Monitor:

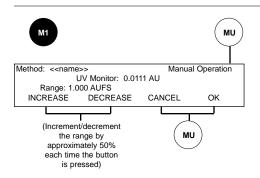




The display shows the following:

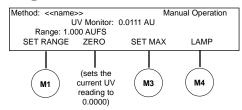
- UV Monitor: The current UV reading.
- Range/Max Range: The chart range for UV detection.

Description of the soft key choices follows.



Set Range: Allows you to increase/decrease the full-scale AU (Absorbance Unit) value for the UV output to the chart recorder. For example, when the range is set to 1.000 AUFS (Absorbance Units Full Scale), the chart recorder pen will be at its highest point ("full scale") when the absorbance of the sample in the flow cell is 1 AU. The following set ranges are available: 2.0, 1.0, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001 AUFS.

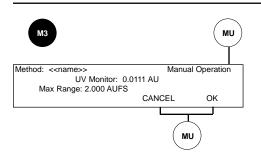




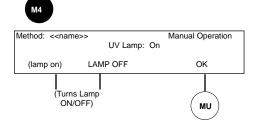
Zero: This allows you to zero the UV baseline on the chart recorder.

Table 9. (continued) Manual Mode Operation: UV Monitor

Reference Description



Set Max: Use this function to set a UV range other than that selectable using **Set Range**. When the UV absorbance reading reaches the maximum range set, the system delivers 1 volt to the chart recorder. Enter a value between 2.0 and .001 AUFS.



Lamp: This turns the UV lamp on/off. You may want to leave the BioLogic LP system ON but shut off the lamp when the system is not in use.

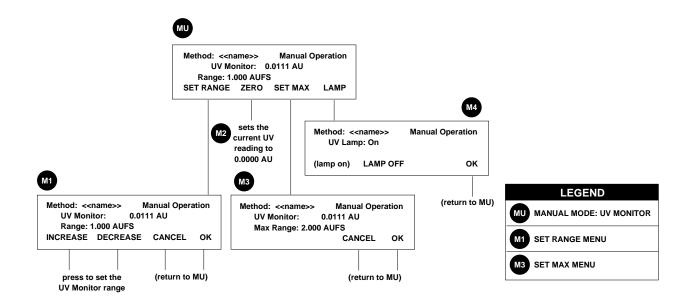


Figure 18. Manual Operation of the UV Monitor

(Screen displays are keyed to the discussion in Table 9, Manual Mode Operation : UV Monitor)

5.2.5 Manual Mode Operation of the Conductivity Monitor

Table 10 discusses the Conductivity Monitor's Manual mode operation. Figure 19 provides an overview.

Table 10.

Manual Mode Operation: Conductivity Monitor

Reference Description

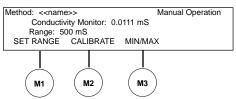




To manually control the Conductivity Monitor:

- a. From the Mode keys select Manual.
- b. From the Instrument keys select Cond.
- c. The main menu for manual control of the Conductivity Monitor is shown below.

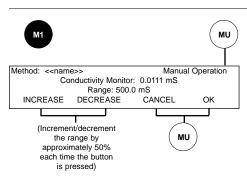




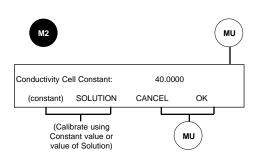
The display shows the following:

- Conductivity Monitor: The current Conductivity reading.
- Range: The chart range for Conductivity detection.

Description of the soft key choices follows.



Set Range: Allows you to increase/decrease the full scale conductivity value (in millisiemens) for the conductivity output to the chart recorder. For example, when the range is set to 100.0 mS (millisiemens), the chart recorder pen will be at its highest ("full scale") when the conductivity of the sample in the flow cell is 100 millisiemens per centimeter. The following Set Ranges are available: 500.0, 200.0, 100.0, 50.0, 20.0, 10.0, 5.0, 1.0, and 0.5 mS.

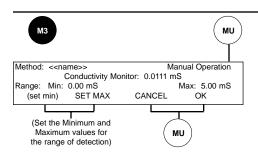


Calibrate: Allows you to calibrate using either of the following values:

- Constant: When a conductivity flow cell is installed for the first time, use this selection to enter its cell constant into the system. Enter a constant value (less than 100) using the numeric keypad. Each cell is labeled with a cell constant. The nominal cell constant is 40.
- Solution: Use this selection to calibrate the system using a solution of known conductivity. (This may be a solution for which conductivity was determined using a benchtop conductivity bridge, or a solution purchased specifically to provide a conductivity standard.) Fill the flow cell with the solution, and then enter its conductivity value. Calibration solutions should be between 10 and 100 mS/cm.

Table 10. (continued) Manual Mode Operation: Conductivity Monitor

Reference Description



Min/Max: Allows you to set a range, from 0.00 mS to 999.99 mS, not selectable under Set Range's incremental values. To use this function, run buffer A through the system until the conductivity reading stabilizes, and note the conductivity reading. Then run buffer B through the system and note its conductivity reading. In the conductivity Min/Max screen, enter the noted A and B values as the minimum and maximum.

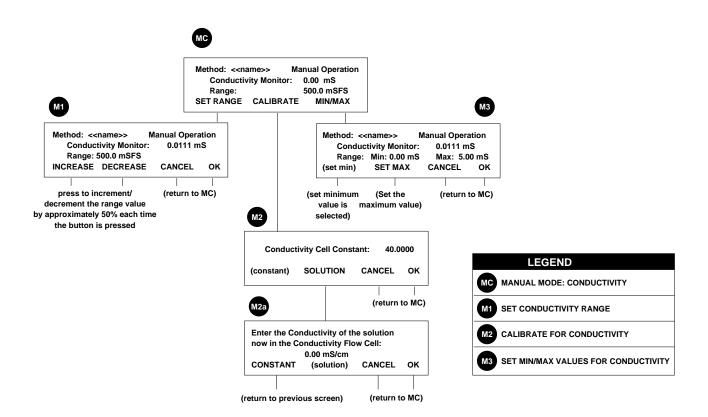


Figure 19. Manual Operation of the Conductivity Monitor

(Screen displays are keyed to the discussion in Table 10, Manual Mode Operation : Conductivity Monitor)

5.2.6 Manual Mode Operation of the Valves

Table 11 discusses each of the functions available in Manual mode operation of the Valves. Figure 20 provides an overview of those functions.

Table 11.
Manual Mode Operation: Valves

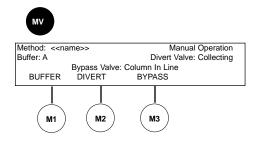
Reference Description





To manually control the Valves:

- a. From the Mode keys select Manual.
- b. From the Instrument keys select Valves.
- The main menu for manual control of the Valves is shown below.

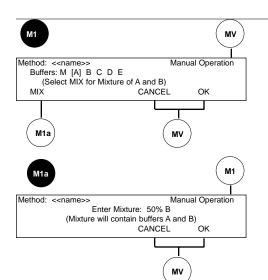


The display shows the following:

- Buffer: The currently selected buffer (A E) or mixture of Buffers A and B.
- Divert Valve: The status of the SV-3 Diverter valve.
- Bypass Valve: The status of the SV-3 Bypass valve.

Description of the soft key choices follows.

Note: If the Divert or Bypass valves are not connected, their softkeys are displayed in lowercase letter and cannot be selected.



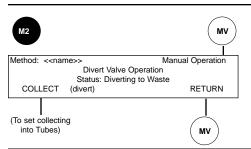
Buffer screen: Allows you to select the buffer delivered to the column. You can select buffers A through E, or a mixture of buffers A and B. Use the **Prev** or **Next** keys to select a buffer; to select a mixture of buffers A and B, press the **MIX** soft key. (Refer to the Mix screen shown below.)

Note: If a valve is not connected to the controller it's letter or letters appear in lower case and cannot be selected.

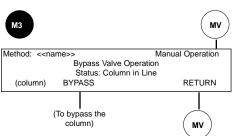
Mix screen: Enter the percentage of Buffer B such that %A + %B = 100%.

Table 11. (continued) Manual Mode Operation: Valves

Reference Description



Divert screen: Allows you to set the SV-3 Diverter valve in either the "Divert to Waste" position or the "Collecting into Tubes" position.



Bypass: Allows you to set the SV-3 Bypass valve in either the "Column in Line" position or the "Bypassing Column" position. Note: The SV-3 Column Bypass valve is operated only in Manual mode; its operation cannot be programmed as part of a Method.

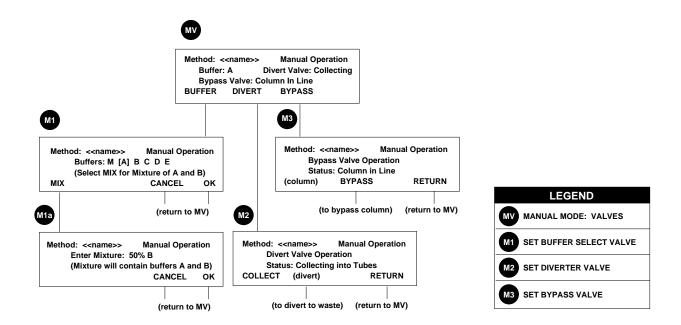


Figure 20. Manual Operation of the Valves

(Screen displays are keyed to the discussion in Table 11, Manual Mode Operation : Valves)

5.2.7 Manual Mode Operation of the Chart Recorder

Table 12 discusses each of the functions available in Manual mode operation of the Chart Recorder.

Table 12.

Manual Mode Operation: Chart Recorder

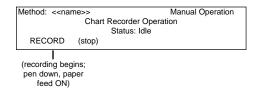
Reference Description





To manually control the Chart Recorder:

- a. From the Mode keys select Manual.
- b. From the Instrument keys select Recorder.



The Chart recorder screen allows you to start and stop the chart recorder. The following selections are available:

- Record: The chart recorder will begin to record data (pen down, paper feed ON).
- Stop: The chart recorder will stop (pen up and paper stopped).

5.3 PROGRAMMING MODE

Programming mode is used to define a sequence of actions which collectively comprise a method. Once a method is written the BioLogic LP can carry out the programmed actions unattended by running the method. To enter the Programming Mode press the Program mode key. When viewing a method, the "Method List" presents all programmed actions in the order in which they will occur when the method is run. The Method List is generated automatically each time a method is created, modified, or recalled from storage (opened). The Method List cannot be created or modified directly. The process of creating or modifying a method consists of completing three separate tables which together comprise the method:

- 1. **Pump Step Table:** This table is used to define all pump parameters throughout the entire method. The table is divided into numbered steps and can contain up to 50 steps. Each step includes a buffer, a duration or length, and a flow rate. The buffer can be a single solution, a mixture of two solutions, or a linear gradient. The duration of a step will be in either units of time (minutes) or volume (ml) depending upon the choice made when the method is created. The flow rate will be in ml/min. To view or make changes to the Pump Step Table press the Pump instrument key.
- 2. Fraction Collector Table: This table is used to define the fraction collection parameters for the entire method. Fraction collection can be programmed in any of four different ways including collecting the entire run ("Collect All"), collecting only when the UV detector signal indicates a peak ("Threshold"), collecting during specific periods in the run only ("Windows"), and a combination of the threshold and windows schemes ("Threshold + Windows"). Collection parameters such as fraction size and collection "windows" will be programmed in units of time (minutes) or volume (ml) depending upon the choice made when the method is created. To view or make changes to the Fraction Collection Table press the Collector instrument key.
 - Note: Fraction collection is not required in a method although most methods will typically include fraction collection. When Windows are used the method may contain up to 50 windows.
- 3. Alarm Table: This table is used to define up to three specific points in the progress of a run when an alarm will sound. If desired, a Hold can be placed at the time of an alarm to stop the progress of the run until the user chooses to continue the run. To view or make changes to the Alarm Table press the Alarm instrument key.
 - Note: Alarms are not required in a method and are only valuable when the user will be nearby during the run.

Figure 21 shows the relationship between the contents of the three tables described above and the Method List.

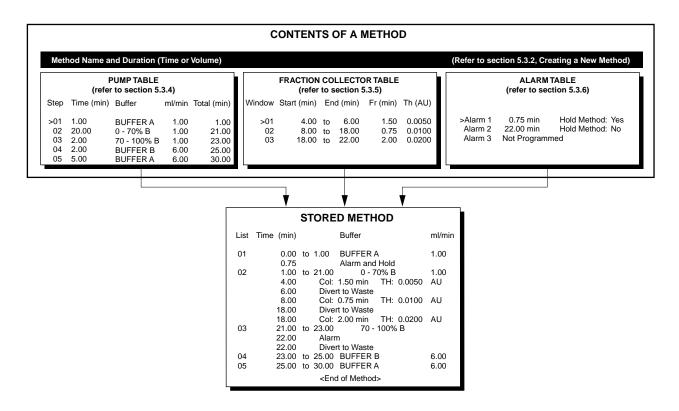


Figure 21. Contents of a Method

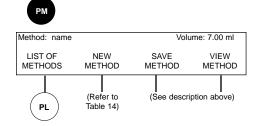
5.3.1 Program Mode's Main Menu

To enter Program mode, press the **Prog** key. The Program display shows the currently opened method, and provides a main menu consisting of four softkey selections:

- **List of Methods**: Selecting this softkey displays the list of stored methods. Use this softkey to select a method to open, rename, or delete a method. For details, refer to Table 13, List of Methods.
- **New Method**: Selecting this softkey allows you to begin creating a new method. For further discussion, refer to section 5.3.2, Creating a New Method.
- **Save Method**: This softkey allows you to enter a name and save a method. For further discussion, refer to section 5.3.7, Entering Method Names.
- **View Method**. This softkey allows you to view the Method List in the currently opened method. To scroll through the list, press the **Prev** and **Next** keys on the front panel.

Table 13.
Program Mode Operation: List of Methods

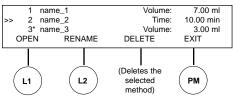
Reference Description



Main Menu screen: The display shows the name of the currently selected method and its total duration.

To display the list of all available methods, select **List of Methods**.





List of Methods screen: This is a list of all methods which are stored in memory along with the duration of the method. The duration of the method is labeled in the units chosen when the method was written. Stored methods are displayed in the list in alphabetical order. The current method is indicated by the asterisk (*). The method which is selected and will be affected by the actions of the soft keys is indicated by the double arrowhead (>>). Use the **Prev** and **Next** keys to scroll through the list and view all of the methods stored.

 Open: This retrieves the selected method from storage and makes it the current method. The display will show the Method List for the selected method. For a description of the Method List and how to use this list see section 5.3.3.

Note: If the current method is not saved when opening another method then it is permanently lost.

Table 13. (continued) Program Mode Operation: List of Methods

Reference Description

- Rename: This allows you to change the method's name.
- Delete: Deletes the selected method. Deleting the open method (indicated by the asterisk), deletes it from the List of Methods, however, it remains the current method as "< untitled >". Deleting any Method other than the open method means it cannot be recovered.
- **Exit**: Returns to programming's main menu screen, without changing the current method.

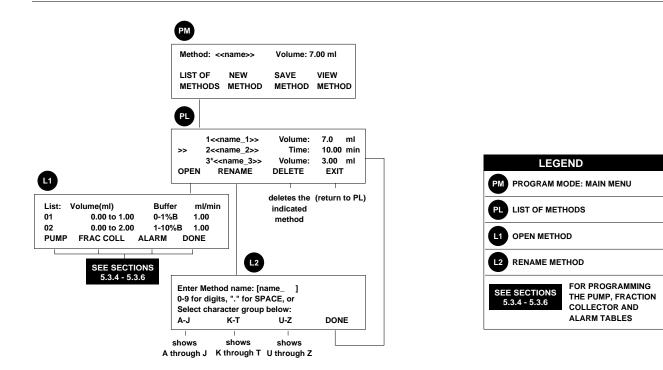


Figure 22. List of Methods

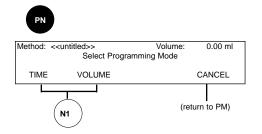
(Screen displays are keyed to the discussion in Table 13, Program Mode Operation : List of Methods)

5.3.2 Creating a New Method

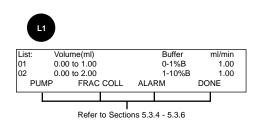
To create a new method enter Programming mode by pressing the **Program** mode key. From the Programming mode main menu the **New Method** softkey is used to create an entirely new method. Note: It is often easier to open a stored method which is similar to the desired new method, modify it as desired, and then save it using a new name. See section 5.3.3 for a description of how to do this.

Table 14.
Program Mode Operation: New Method

Reference Description



Select Time or Volume mode: The units used to define the actions within a method can be either time (minutes) or volume (ml). Once this selection is made the method cannot be changed from one unit type to the other unit type. After this selection is made the method actions are programmed starting with the pump steps. For a description of programming pump steps see section 5.3.4. When all pump steps have been programmed, the **OK** softkey displays the **Method List** containing only pump steps.



Method List: This list shows the actions which will take place when the method is run in the order in which they will occur. The numbered steps indicate the pump steps within the method. Use the **Prev** and **Next** keys to scroll through the list and view the method. The units of duration are in either time (minutes) or volume (ml) depending upon the choice made when the method was created.

If additional pump steps or changes to the pump steps are desired see section 5.3.4. If fraction collection is desired during this method see section 5.3.5 which describes fraction collection programming. If Alarms are desired within the method see section 5.3.6 which describes alarm programming.

After each table is completed the Method List will be displayed again including the changes made and can be reviewed . You will notice that the list shows fraction collection and alarms as events and places them directly under the pump step in which they occur. When all desired actions have been programmed and the method is complete, press the **Done** softkey.

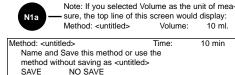
Notes: A method must contain at least one pump step; however fraction collection and alarms are optional. The other BioLogic LP instruments (UV Monitor, Conductivity Monitor, Chart Recorder, and Valve Controller) are not part of programmed methods. They can be operated by pressing the

Table 13. (continued) Program Mode Operation: New Method

Description Reference

10 min

appropriate instrument key at any time, except while actually viewing and editing a method. Although the Chart Recorder is not programmed, it automatically records all runs.



PM

(Enter Method's name before returning to PM. For further discussion refer to discussion at start of section 5.3.7.)

Save Method screen: This screen allows the following choic-

Save: This allows a name to be entered for the method and the method to be saved in permanent storage. For a description of how to enter method names see section 5.3.7.

Note: All methods which have names other than "<untitled>" have been saved in permanent storage.

No Save: This allows the method to remain the current method and for it to be run without saving it in permanent storage. This option is typically used when a method will never be repeated or when 50 methods are already stored in permanent memory. A method which is not saved will always be named "<untitled>".

Note: The "<untitled>" method loaded as the current method is not lost when the power is shut off and remains the current method when power is restored.

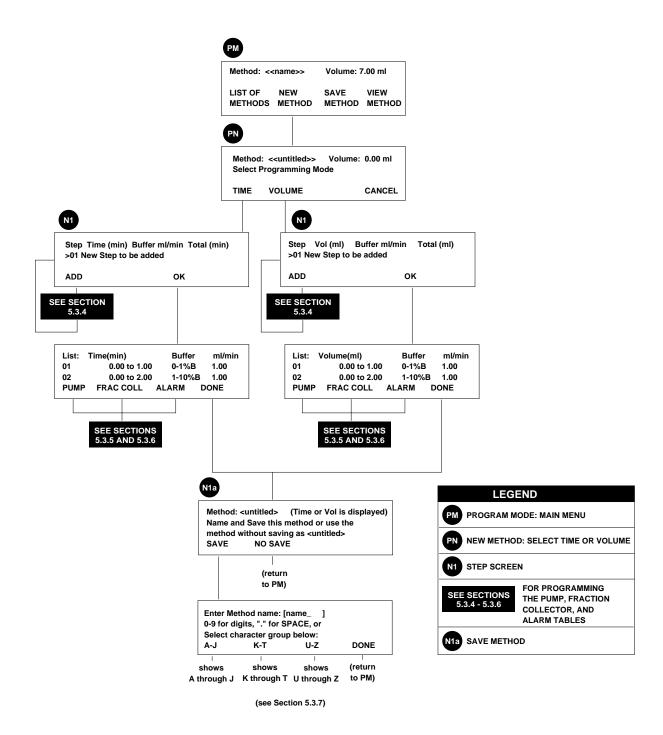


Figure 23. Programming a New Method

(Screen displays are keyed to the discussion in Table 14, Program Mode Operation: New Method)

Reference

5.3.3 Viewing and Editing a Method

Whenever a method is Opened, or whenever View Method is selected, the Method List is displayed. From the Method List it is possible to review the contents of the entire method including the pump steps, fraction collection parameters, and the alarms. If desired, the method can be edited to make whatever changes are necessary. When the contents of the method are correct, press the **Done** softkey. If changes are made, you must then confirm whether or not you want to save the method. Refer to section 5.3.7 for saving the method. The system then returns to the Program mode main menu. To run the method, see section 5.4.

Table 15. Program Mode Operation: Viewing and Editing a Method

Description

Method List: This list shows all actions which will take place when the method is run in the order in which they will occur. Accessed from any of the following Program mode main menu selections: The numbered steps indicate the pump steps within the List of Methods method. Below each step are any fraction collection events or New Method View Method alarm events which will occur during the step. Use the Prev and **Next** keys to scroll through the list and view the method. Volume(ml) Buffer ml/min 0-1%B 0.00 to 1.00 1.00 The units of duration are in either time (minutes) or volume (ml) 0.00 to 2.00 1-10%B 1.00 PLIMP FRAC COLL ALARM DONE depending upon the choice made when the method was created.

> The instrument softkeys or the corresponding instrument keys are used to edit or make changes to the table for the appropriate instrument. For a description of the Pump Table see section 5.3.4. For a description of the Fraction Collection Table see section 5.3.5. For a description of the Alarm Table see section 5.3.6. After the tables are completed the Method List will be displayed again including any changes made and can be reviewed. When the method is complete, press the **Done** softkey. If the method has only been viewed without making any changes the Program mode Main Menu will be displayed. If any changes or additions have been made to the method the Save As menu will be displayed.

Notes: A method must contain at least one pump step; however fraction collection and alarms are optional. The other BioLogic LP instruments (UV Monitor, Conductivity Monitor, Chart Recorder, and Valve Controller) are not part of programmed methods. They can be operated by pressing the appropriate instrument key at any time, except while actually viewing and editing a method. Although the Chart Recorder is not programmed, it automatically records all runs.

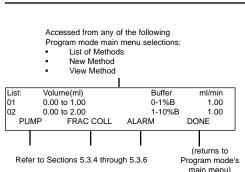
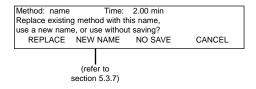


Table 15. (continued Program Mode Operation: Viewing and Editing a Method

Reference Description



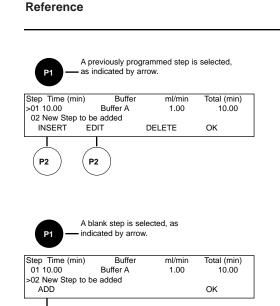
Save As... screen: This screen provides a number of options.

- Replace: This replaces the previous version of a method with the newly modified method. In this case the previous version is permanently lost.
- New Name: This allows the modified method to be saved using a new name. In this case both the original method and the modified method with a different name are saved.
- No Save: This allows the method to remain the current method and for it to be run without saving it in permanent storage. This option is typically used when a method will never be repeated or when 50 methods are already stored in permanent memory. Methods which are not saved will always be named "<untitled>".
 - Note: The "<untitled>" method loaded as the current method is not lost when the power is shut off and remains the current method when power is restored.
- Cancel: This gives the option to abandon the changes or additions to the original method and revert to the method as it was before. There will be a confirmation screen with a Yes/No choice to prevent accidental loss of the modified method if this key is pressed unintentionally.

5.3.4 Program Mode's Pump Table

The Pump Table displays the contents of all pump steps within the method. This table can be viewed by pressing the Pump instrument key while in Program mode or by pressing the Pump softkey on the Method List screen. Each pump step contains a buffer composition, a duration, and a flow rate. Table 16 describes how to program the Pump Table.

Table 16.
Program Mode's Pump Table



Pump Table: Each step in a method is shown in a scrolling list format. The selected step is shown with an arrow (>) pointing to the step. Use the **Prev** and **Next** keys to move the selection up and down the list. When the selected step is already programmed, the softkeys are as shown in the top picture and when the selected step is not already programmed the softkeys are as shown in the bottom picture.

Description

The table consists of 5 columns: the step number, the duration, the buffer, the flow rate, and the cumulative method duration including the step. Up to 50 steps can be programmed in a method and the duration units will be either time (minutes) or volume (ml), depending upon the choice made when the method was created.

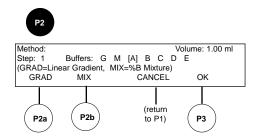
- Insert: This places a new step into the method, which will have the same step number as the current selected step. The currently selected step and all subsequent steps will have their step number increased by one.
- Edit: This allows the contents of the selected step to be changed.
- **Delete**: This removes the selected step from the table. The step cannot be retrieved.
- Add: This allows a new step to be added to the end of the table.
- **OK**: This indicates that the table is complete and returns to the Method List.

Note: To program the Fraction Collector Table or the Alarm Table without returning to the Method List press the **Collector** and **Alarm** keys respectively while viewing the Pump Table.

P2

Table 16. (continued) Program Mode's Pump Table

Reference Description



Select Buffer: The buffers available are indicated by the letters A through E plus the letters G and M which are shown on line 2 of the LCD. If a buffer is available the letter will be an upper case letter. When a buffer is not available it is shown in a lower case letter.

The availability of buffers is controlled by the presence or absence of the system valves. Buffer A is always available. Buffer B becomes available when the Proportioning Valve/Mixer is plugged into the Controller. Buffer C becomes available when an SV3-2 valve is plugged into the Buffer Selector port. Buffers C, D, and E become available when an SV5-4 valve is plugged into the Buffer Selector port. The Controller senses the presence of the valves and automatically updates this list as valves are plugged and unplugged from the Controller.

For convenience, all buffers can be programmed in a method even when not currently available. To run the method the appropriate valves must be connected.

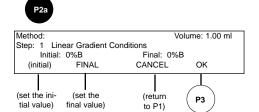
The letters G and M stand for Gradient and Mixture. Gradient refers to a linear gradient made from buffers A and B and Mixture refers to a single solution made by mixing buffers A and B. Gradient and Mixture compositions are expressed in %B. The brackets around one of the buffers, [A], indicate which buffer is currently selected. Use the **Prev** and **Next** keys to change the selection.

- Grad: This is used to program a linear gradient in this step.
- Mix: This is used to program a mixture in this step.
- Cancel: This cancels the addition or modification of this step and returns to the Pump Table as it was originally.
- OK: This confirms the selected buffer as indicated by the brackets surrounding the letter and continues to the next entry.

Note: The Grad and Mix softkeys are identical to selecting the G and the M respectively from the list.

Table 16. (continued) Program Mode's Pump Table

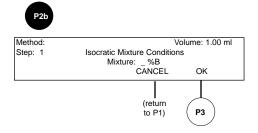
Reference Description



Linear Gradient: A linear gradient consists of a continuous change in the buffer composition from an initial mixture of buffers A and B to a final mixture of buffers A and B. Buffer composition is set in %B such that %A + %B = 100%.

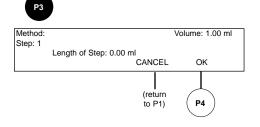
- Initial: This places the cursor in the field to enter buffer composition at the start of the gradient. Enter the value using the number keys.
- Final: This places the cursor in the field to enter buffer composition at the end of the gradient. Enter the value using the number keys.
- **Cancel**: This cancels the addition or modification of this step and returns to the Pump Table as it was originally.
- OK: This confirms the linear gradient entered and continues to the step duration entry.

Note: The **Prev** and **Next** keys also move the cursor between the initial and final values.



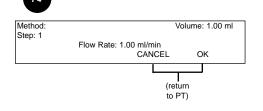
Mixture: A mixture continuously delivers a combination of buffers A and B. This delivery is isocratic or constant throughout the step. The buffer composition is set in %B such that %A + %B = 100%. Enter the value using the number keys.

- Cancel: This cancels the addition or modification of this step and returns to the Pump Table as it was originally.
- OK: This confirms the isocratic mixture entered and continues to the step duration entry.



Length of Step: The duration of this step is entered using the number keys. The duration is in either units of time(minutes) or volume (ml) depending upon the choice made when the method was created.

- Cancel: This cancels the addition or modification of this step and returns to the Pump Table as it was originally.
- **OK**: This confirms the length of step entered and continues to the step flow rate entry.



Flow Rate: The flow rate of this step is entered using the number keys. The flow rate entry is the final entry for a pump step.

- Cancel: This cancels the addition or modification of this step and returns to the Pump Table as it was originally.
- **OK**: This confirms the flow rate entered thereby completing the step and returns to the Pump Table with the new or modified step included in the table.

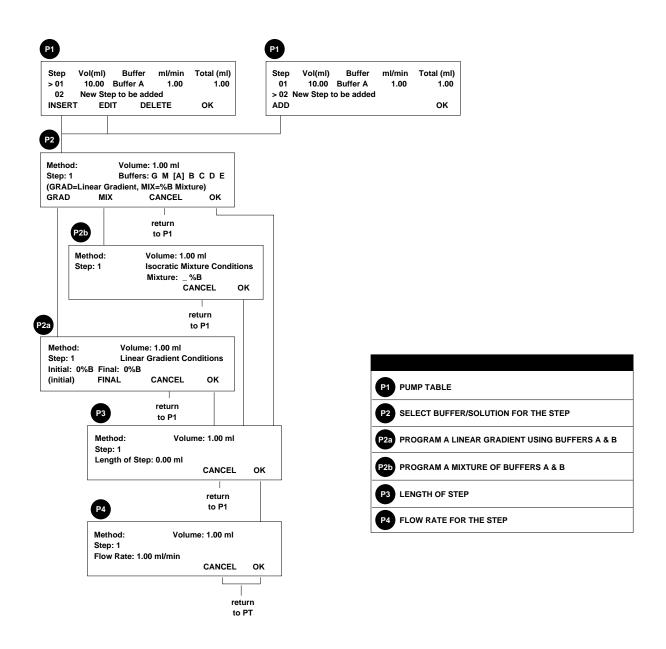


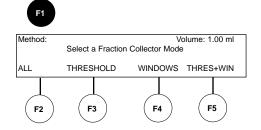
Figure 24. Programming: the Pump Table (Screen displays are keyed to the discussion in Table 16)

5.3.5 Program Mode's Fraction Collector Table

The Fraction Collector Table displays the fraction collector parameters within the method. This table is viewed upon the completion of selecting a collection mode and entering the necessary parameters. To begin this process press the **Collector** instrument key while in Program mode or press the **Frac Coll** softkey on the Method List screen. If fraction collection has not been programmed within the method, the Select Collection Mode screen will be presented. There are four different fraction collection modes which can be used: Collect All, Threshold Collection, Collection Windows, and Threshold plus Collection Windows. Each of these modes will be discussed individually below. If a fraction collection mode was previously programmed within the method, then the Fraction Collection Summary screen will be presented.

Table 17.
Program Mode's Fraction Collector Table

Reference Description



Select Collection Mode: The fraction collection mode is chosen using the softkeys. There must be an SV3-2 valve (or System Cable 3 with a Model 2128) plugged into the Diverter Valve port on the Controller to run a method using any collection mode except Collect All.* After making a selection, the parameter entry screen(s) for that collection mode will be presented. For descriptions of these screens consult the appropriate sections below.

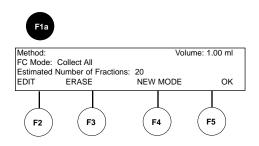
Note: If no fraction collection is desired use the Pump key or Alarm key to leave this screen without making a selection.

- All: Selects Collect All mode which collects fractions throughout the entire method. See section 5.3.5.1.
- Threshold: Selects Threshold Collection mode which allows you to only collect peaks by defining a threshold value in absorbance units (AU). See section 5.3.5.2.
- Windows: Selects Collection Windows mode allows you to specify periods called "windows" within the method when fractions will be collected. See section 5.3.5.3.
- Thresh+Win: Selects Threshold plus Collection Windows mode is a combination of the two modes described above. See section 5.3.5.4.

*For convenience, all collection modes can be programmed without the valve connected.

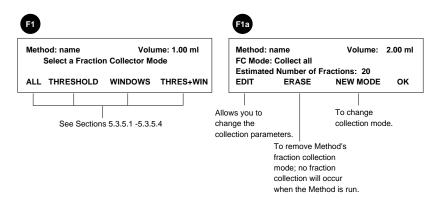
Table 17. (continued) Program Mode's Fraction Collector Table

Reference Description



Fraction Collection Summary: This screen shows the current collection mode and an estimate of the number of fractions which will be collected when the method is run. The estimate is accurate when threshold collection is not used and no manual overrides are performed during the run. This estimate will be incorrect if manual overrides are performed during the run or when a threshold is used. In either case the actual number of fractions collected can be either more or less than the estimate.

- **Edit**: Allows the fraction collection parameters to be viewed and changed.
- Erase: Removes the existing fraction collection programming from the method. No fraction will occur when the method is run.
- New Mode: Allows the collection mode to be changed by displaying the Select Collection Mode screen.
- OK: Returns to the Method List



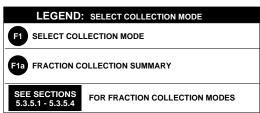


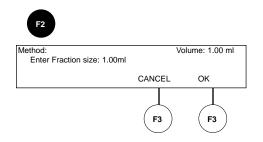
Figure 25. Programming: the Fraction Collector Table's Summary (Screen displays are keyed to the discussion in Table 17)

5.3.5.1 Collect All Mode

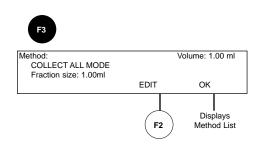
Collect All mode uses a single fraction size to collect fractions throughout a method. The units used for the fraction size are either time (minutes) or volume (ml), depending upon the choice made when the method was created. Table 18 describes how to program Collect All Mode.

Table 18.
Program Mode's Fraction Collector Table: Collect All

Reference Description



Fraction Size: Enter the fraction size desired using the number keys. The **OK** softkey confirms the fraction size entered. The **Cancel** softkey reverts to the original or the default fraction size. Both softkeys display the Fraction Collection Table for Collect All mode.



Fraction Collection Table for Collect All mode: This table shows the current fraction mode and the collection parameters. To change the parameters press the **Edit** softkey. To display the Method List press the OK softkey.

Note: To program the Pump Table or the Alarm Table without returning to the Method List, press the **Pump** and **Alarm** keys respectively while viewing the Fraction Collector Table.

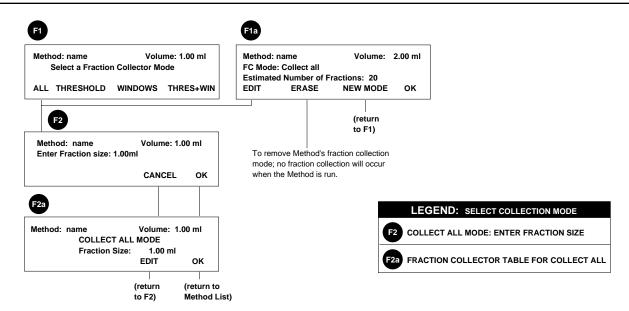


Figure 26. Programming: the Fraction Collector Table's Collect All (Screen displays are keyed to the discussion in Table 18)

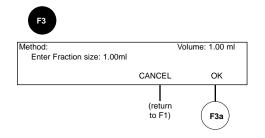
5.3.5.2 Threshold Collection Mode

Threshold Collection mode allows you to only collect peaks by defining a threshold value in absorbance units (AU). When the absorbance is above the threshold value the Diverter Valve sends the fluid to the fraction collector and the collector advances as programmed. When the absorbance is below the threshold value the Diverter Valve sends the fluid to waste. To prevent air bubbles and noise from being collected as peaks there is an optional Bubble Filter. This filter can be set to High, Medium, Low, or Off. As the setting is increased broader peaks are considered air bubbles or noise and are not collected. When the filter is Off, all peaks are collected. The units used for the fraction size are either time (minutes) or volume (ml), depending upon the choice made when the method was created. Table 19 describes how to program Threshold Mode.

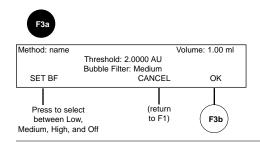
Table 19.

Program Mode's Fraction Collector Table: Threshold Collection

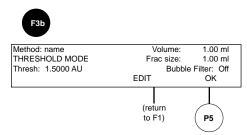
Reference Description



Fraction Size: Enter the fraction size desired using the number keys. The **OK** softkey confirms the fraction size entered and continues to the threshold and bubble filter entry screen. The **Cancel** softkey displays the **Select Collection Mode** screen.



Threshold and Bubble Filter: Enter the threshold using the number keys. The **Set BF** softkey will increment the bubble filter setting through a circular list. The **OK** softkey confirms the threshold and bubble filter entered and softkeys display the Fraction Collection Table for Threshold mode. The **Cancel** softkey displays the Select Collection Mode screen.



Fraction Collection Table for Threshold mode: This table shows the current fraction mode and the collection parameters. To change the parameters press the **Edit** softkey. To display the Method List press the **OK** softkey.

Note: To program the Pump Table or the Alarm Table without returning to the Method List press the **Pump** and **Alarm** keys respectively while viewing the Fraction Collector Table.

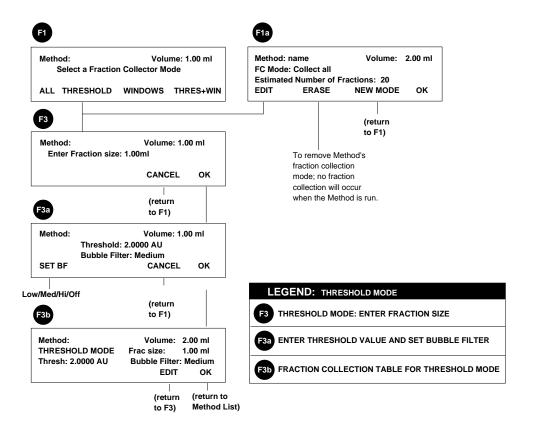


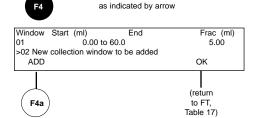
Figure 27. Programming: the Fraction Collector's Threshold Mode (Screen displays are keyed to the discussion in Table 19)

5.3.5.3 Collection Windows Mode

Collection Windows mode allows you to specify periods called "windows" within the method when fractions will be collected. Each window can have a different fraction size. During a window the Diverter Valve sends the fluid to the fraction collector and the collector advances as programmed. Outside of the windows the Diverter Valve sends the fluid to waste. The units used for the fraction size and Collection Windows are either time (minutes) or volume (ml) depending upon the choice made when the method was created. Table 20 describes how to program Collection Windows Mode.

Table 20.
Program Mode's Fraction Collector Table: Collection Windows

Reference A previously programmed Window is selected, as indicated by arrow Window Start (ml) Frac (ml) 0.00 to 60.0 5.00 02 New collection window to be added **INSERT** EDIT DELETE OK (return F4a F4a to FT, Table 17)



A blank Window is selected,

Description

Fraction Collector Table for Collection Windows Mode:

Each window in a method is shown in a scrolling list format. The selected window is shown with an arrow (>) pointing to the window. Use the **Prev** and **Next** keys to move the selection up and down the list.

When the selected window is already programmed the softkeys are as shown in the top picture and when the selected window is not already programmed the softkeys are as shown in the bottom picture.

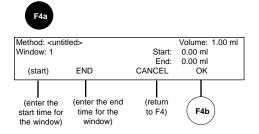
The table consists of 4 columns: the window number, the start of the window, the end of the window, and the fraction size. Up to 50 windows can be programmed in a method and the units will be either time (minutes) or volume (ml) depending upon the choice made when the method was created.

- Insert: This places a new window into the method using the window number of the current selected window. The currently selected window and all subsequent windows have their window number increased by one.
- **Edit**: This allows the contents of the selected window to be changed.
- **Delete**: This removes the selected window from the table. The window cannot be retrieved.
- Add: This allows a new window to be added to the end of the table.
- OK: This indicates that the table is complete and returns to the Method List.

Note: To program the Pump Table or the Alarm Table without returning to the Method List press the **Pump** and **Alarm** keys respectively while viewing the Fraction Collector Table.

Table 20. (continued) Program Mode's Fraction Collector Table: Collection Windows

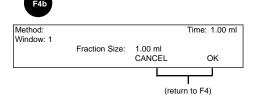
Reference Description



Window Start and End: Enter the times or volumes when the collection window should occur within the method.

- **Start**: This places the cursor in the field to enter the start of the window. Enter the value using the number keys.
- **Final**: This places the cursor in the field to enter the end of the window. Enter the value using the number keys.
- Cancel: This cancels the addition or modification of this window and returns to the Fraction Collector Table as it was originally.
- **OK**: This confirms the start and end values entered and continues to the fraction size entry.

Note: The **Prev** and **Next** keys also move the cursor between the start and end values.



Fraction Size: Enter the fraction size desired using the number keys. The **OK** softkey confirms the fraction size entered and returns to the Fraction Collector Table with the new or modified window in the table. The **Cancel** softkey returns to the Fraction Collector Table as it was originally without adding or modifying the window.

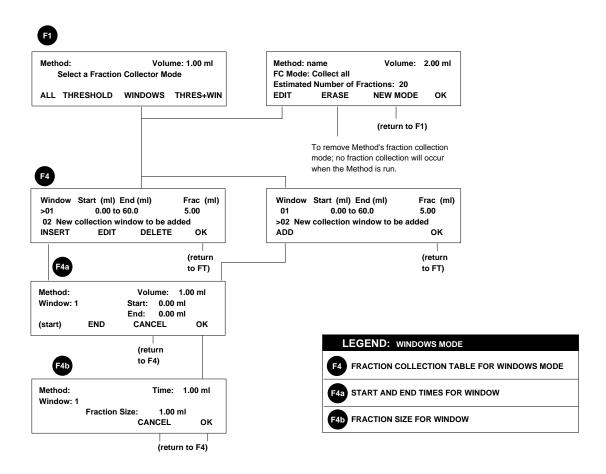


Figure 28. Programming: the Fraction Collector's Windows Mode (Screen displays are keyed to the discussion in Table 20)

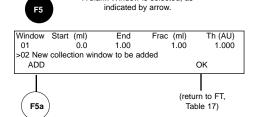
5.3.5.4 Threshold and Collection Windows Collection Mode

Threshold plus Collection Windows mode is a combination of Threshold Collection Mode and Collection Windows Mode. The description of this mode assumes a complete understanding of the two individual modes which are described in sections 5.3.5.2 and 5.3.5.3.

In Threshold plus Collection Windows Mode each window can have a different threshold as well as a different fraction size. In this mode the Diverter Valve sends the fluid to the collector *only when both* inside a collection window *and* above the threshold for the window. In all other circumstances the fluid is sent to waste. The units used for the fraction size and collection windows are either time (minutes) or volume (ml) depending upon the choice made when the method was created. Table 21 describes how to program Threshold plus Collection Windows Mode.

Table 21.
Program Mode's Fraction Collector Table: Threshold with Collection Windows

Reference Description A previously Programmed Window is selected, as **E**5 indicated by arrow. Window Start (ml) Frac (ml) Th (AU) 0.0 1.00 1.00 1.000 02 New collection window to be added EDIT DELETE OK INSERT (return to FT. F5a F5a Table 17)



A blank Window is selected, as

Fraction Collector Table for Threshold plus Collection Windows Mode: Each window in a method is shown in a scrolling list format. The selected window is shown with an arrow (>) pointing to the window. Use the Prev and Next keys to move the selection up and down the list.

When the selected window is already programmed, the softkeys are as shown in the top picture; and when the selected window is not already programmed, the softkeys are as shown in the bottom picture.

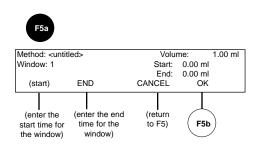
The table consists of 5 columns: the window number, the start of the window, the end of the window, the fraction size, and the threshold. Up to 50 windows can be programmed in a method, and the units will be either time (minutes) or volume (ml), depending on the choice made when the method was created.

- Insert: This places a new window into the method using the window number of the current selected window. The currently selected window and all subsequent windows have their window number increased by one.
- Edit: This allows the contents of the selected window to be changed.
- Delete: This removes the selected window from the table.
 The window cannot be retrieved.
- Add: This allows a new window to be added to the end of the table.
- OK: This indicates that the table is complete and returns to the Method List.

Note: To program the Pump Table or the Alarm Table without returning to the Method List, press the **Pump** and **Alarm** keys respectively while viewing the Fraction Collector Table.

Table 21. (continued) Program Mode's Fraction Collector Table: Threshold with Collection Windows

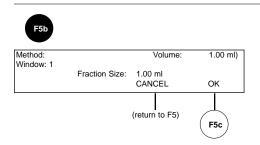
Reference Description



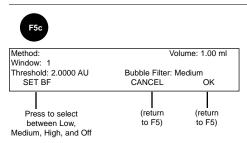
Window Start and End: Enter the times or volumes when the collection window should occur within the method.

- **Start**: This places the cursor in the field to enter the start of the window. Enter the value using the number keys.
- **Final**: This places the cursor in the field to enter the end of the window. Enter the value using the number keys.
- Cancel: This cancels the addition or modification of this window and returns to the Fraction Collector Table as it was originally.
- OK: This confirms the start and end values entered and continues to the fraction size entry.

Note: The **Prev** and **Next** keys also move the cursor between the start and end values.



Fraction Size: Enter the fraction size desired using the number keys. The **OK** softkey confirms the fraction size entered and continues to the threshold and bubble filter entry screen. The **Cancel** softkey returns to the Fraction Collector Table as it was originally, without adding or modifying the window.



Threshold and Bubble Filter: Enter the threshold using the number keys. The Set BF softkey will increment the bubble filter setting through a circular list. The OK softkey confirms the threshold and bubble filter entered and returns to the Fraction Collector Table with the new or modified window in the table. The Cancel softkey returns to the Fraction Collector Table as it was originally, without adding or modifying the window.

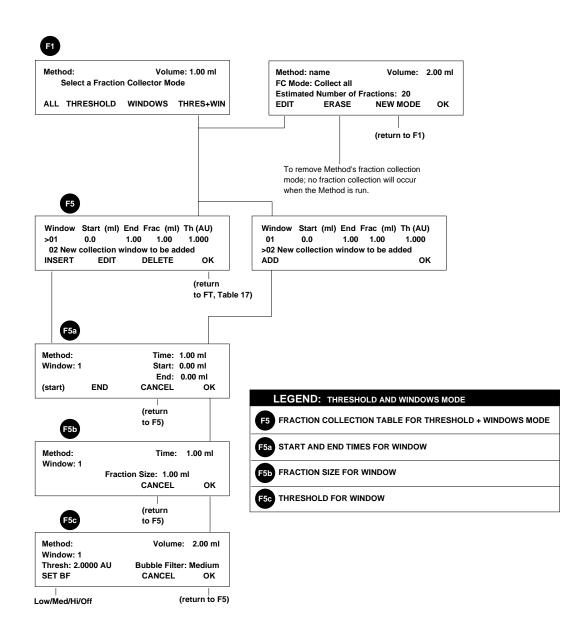


Figure 29. Programming: the Fraction Collector's Threshold with Collection Windows Mode (Screen displays are keyed to the discussion in Table 21)

5.3.6 Program Mode's Alarm Table

The Alarm Table displays the alarms programmed within the method. This table can be viewed by pressing the **Alarm** instrument key while in Program mode or by pressing the **Alarm** softkey on the Method List screen. When an alarm occurs the alarm status light will turn on, the **Alarm** instrument light will flash, and a beeper will sound. The first key pressed when an alarm occurs will not have it's normal effect but will instead simply cancel the alarm. As an option the method can be placed on Hold when an alarm occurs. When a method is held, the run timer stops counting but the pump continues to pump the current buffer. All other instruments continue to operate during this time as well. Holds last until the user "Continues" the run. For more information about holding a run see section 5.4.2.2. The units used for the alarms are either time (minutes) or volume (ml) depending upon the choice made when the method was created. Table 22 describes how to program the Alarm Table.

Table 22.
Program Mode's Alarm Table

Reference A previously Programmed Alarm is **A1** selected, as indicated by the arrow. >Alarm 1 Hold Method: Yes Not Programmed Alarm 2 Not Programmed Alarm 3 DELETE OK (return to Method List) A1a A Blank Alarm is selected, Hold Method: Yes 10 min Alarm 2 Not Programmed Alarm 3 Not Programmed ADD

(return to Method List)

Alarm Table: Each alarm in a method is shown in a scrolling list format. The selected alarm is shown with an arrow (>) pointing to the alarm. Use the **Prev** and **Next** keys to move the selection up and down the list.

Description

When the selected alarm is already programmed, the softkeys are as shown in the top picture; and when the selected alarm is not already programmed, the softkeys are as shown in the bottom picture. The table consists of 2 columns: the alarm time or volume and the Hold choice made for the alarm.

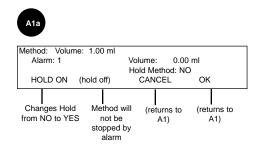
Up to 3 alarms can be programmed in a method, and the units will be either time (minutes) or volume (ml), depending upon the choice made when the method was created. Alarms do not have to be entered in the order in which they will occur. The next time the Alarm Table is viewed, the alarms will be rearranged in order of occurrence.

- Edit: This allows the parameters of the selected alarm to be changed
- Delete: This removes the selected alarm from the table.
 The alarm cannot be retrieved.
- Add: This allows a new alarm to be added to the end of the table.
- OK: This indicates that the table is complete and returns to the Method List.

Note: To program the Pump Table or the Fraction Collector Table without returning to the Method List, press the **Pump** and **Collector** keys respectively while viewing the Alarm Table.

Table 22. (continued) Program Mode's Alarm Table

Reference Description



Alarm Settings: Enter the time or volume for the alarm to occur using the number keys.

- Hold On: This programs the method to Hold when the alarm occurs.
- Hold Off: This programs the method run normally when the alarm occurs.
- Cancel: This returns to the Alarm Table without added or modifying the alarm.
- **OK**: This returns to the Alarm Table with the new or modified alarm in the table.

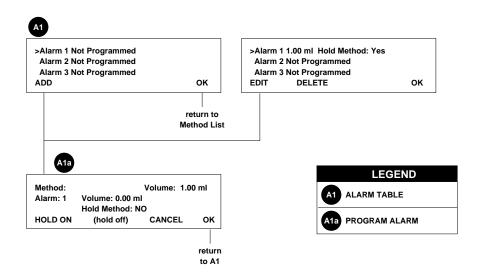


Figure 30. Programming: the Alarm Table (Screen displays are keyed to the discussion in Table 22)

5.3.7 Entering Method Names

Since up to 50 methods can be stored in the BioLogic LP, method names are necessary to keep track of the methods stored. Method names consist of up to 12 characters and can include upper case letters (A-Z), numbers (0-9), and spaces. Careful selection of names makes it easier to retrieve the method desired. In Manual and Run modes the name of the current method will be on the top line of the LCD as a reminder.

Note: The List of Methods used to retrieve stored methods is displayed in alphabetical order.

Table 23.
Program Mode: Entering Method Names

Reference Description A letter range has not been selected. Method Name Entry screen: This screen allows method names to be entered. The underscore shown within the method Enter Method name: [ION EXCH 12_] 0 - 9 for digits " " for SPACE or name on the top line is the insertion point where the next char-Select character group below: acter will be placed. The insertion point can be moved using U-Z DONE the **Prev** and **Next** keys. (finishes (Displays the characters within the indicated range) name entry) To enter a letter character, select the softkey which shows the range containing the desired letter followed by the number key indicated after selecting the range. To enter a number, press Letter range A-J has the number key directly. Use the decimal (.) key to place a been selected. space in the name. Enter Method name: [ION EXCH 12_] A=1 B=2 C=3 D=4 E=5 F=6 G=7 H=8 I=9 J=0 Use SOFT keys to change character groups Line 2 of the LCD displays the current function of each number U-Z A-J key. Press the **Done** soft key to return to the **List of Methods** after the name has been entered. L2 (Displays the characters within the indicated range) Note: The bottom picture shows that after a letter range has been selected the **Done** softkey is replaced by a **0-9** softkey

which can be used to cancel the range selection.

5.4 RUN MODE

Run mode is used to carry out the sequence of actions which are programmed within the current method. The name of the current method which can be run is shown on the top line of the LCD in Manual mode and on the main menu of Program mode. To run a method which is not the current method, see section 5.3 Programming Mode, and section 5.3.1 Programming Mode's Main Menu. The following sections describe starting runs, runs in progress, and interpreting the chart recorder trace.

5.4.1 Starting a Run

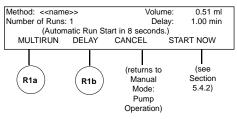
To start the Run Mode press the **Run** mode key. When entering Run mode, an internal system check is made to make certain that all of the required valves are connected and that all run parameters are valid. Section 5.4.1.1 describes the possible errors and how to resolve them and allow the method to be run. Table 24 describes the Run Mode.

Note: The internal check before each run cannot determine if a fraction collector or chart recorder are connected and will assume that they are connected. To avoid problems, check these components before starting a run.

Table 24. Run Mode: Starting a Run

Reference Description





Countdown screen: When entering Run mode, the start of the run is delayed 10 seconds to provide the opportunity to set the Multirun and Delay features. The current status of these features is shown on line 2 of the LCD.

The Multirun setting is always set to 1 when entering Run mode. The Delay setting is remembered between runs so it is important to check this setting. When the Multirun or Delay softkeys are pressed the countdown is stopped while editing the appropriate parameter.

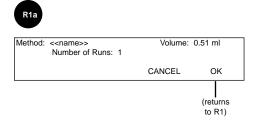
After the parameter is set, the countdown timer is reset to 10 seconds and begins counting again. The run automatically starts when the countdown expires.

- **Multirun**: This allows number of runs to be set or changed.
- Delay: This allows the Delay to be set or changed.
- Cancel: This cancels the run and places the BioLogic LP in Manual mode with the Pump instrument active.
- Start Now: This bypasses the countdown and starts the run immediately.

Note: When entering Run mode, if you do not want to use multiple runs and you are certain that the delay setting is correct, pressing the **Run** mode key two times will bypass the countdown exactly like pressing the **Start Now** softkey.

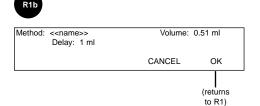
Table 24. (continued) Run Mode: Starting a Run

Reference Description



Multiruns: This allows the method to be run the method from 1 to 999 times. Enter the number of runs desired using the number keys. The **OK** softkey returns to the Countdown screen with the number of runs entered. The **Cancel** softkey returns to the Countdown screen with the original number of runs prior to selecting Multiruns.

Note: The Multirun feature requires a fraction collector capacity of the number of tubes for each run times the number of runs. For example, if 25 fractions are to be collected in each run and Multirun is set to 3 then $25 \times 3 = 75$ fractions will be collected.



Delay: This allows the delay setting to be entered with a value between 0 and the smallest fraction size programmed within the method. Enter the delay setting desired using the number keys. The **OK** softkey returns to the Countdown screen with the delay setting entered. The **Cancel** softkey returns to the Countdown screen with the original delay setting prior to selecting Delay.

Note: For a description of the Delay feature see section 5.4.1.2 below.

5.4.1.1 Errors Which Prevent the Start of Runs

The errors listed below can prevent a method from being run.

- A Required Valve is Missing: A valve which is necessary for the method to run is not present. To
 resolve this error the valve indicated must be connected to the Controller, or the method must be
 edited to omit any use of the valve indicated. The OK softkey returns to Manual Mode with the
 Pump active.
- The Flow Rate Exceeds Pump Calibration: The flow rate programmed within at least one step in the method cannot be reached with the current tubing and calibration. To resolve this error, one or more of the following must be done:
 - 1) the pump tubing must be changed,
 - 2) the pump must be recalibrated, and/or
 - 3) the flow rate within the method must be reduced.

The **OK** softkey returns to Manual Mode with the Pump active.

- The Delay Exceeds the Fraction Size: The current Delay setting is larger than the smallest Fraction Size within the method. To resolve this error, either the delay setting must be reduced or the fraction size must be increased. The **Change** softkey will display the Delay entry screen. The **Cancel** softkey cancels the start of the run and returns to Manual Mode with the Pump active.
- The UV Lamp is Not On: The UV Lamp was turned off. The Change softkey turns on the lamp and returns to the Countdown screen. The **Ignore** key leaves the lamp off and proceeds to the Countdown screen. If the lamp remains off, the UV trace on the chart recorder will not reflect actual run data; however the event marks will still be drawn.
- The Bypass Valve is set to Bypass: The Bypass Valve is currently set to bypass the column. The
 Change softkey changes the valve to the Column position and proceeds to the Countdown screen.
 The Ignore softkey leaves the valve in the Bypass position and proceeds to the Countdown screen.
 The Ignore option allows the Bypass Valve to be used for some other purpose if desired.

Note: The BioLogic LP will run a method even if the UV Monitor Flow Cell and Conductivity Monitor Flow Cell are not connected to the Controller. If either of these are not connected then the chart recorder trace(s) will not reflect actual run data although the UV trace will show the event marks. The BioLogic LP cannot determine if a fraction collector or chart recorder are connected and will assume that they are connected. To avoid problems, check these components before starting a run.

5.4.1.2 Using the Delay Feature

The Delay feature is used to coordinate the UV Monitor signal and event marks on the chart recorder with the fractions collected. The fluid in the UV Monitor flow cell does not actually reach the fraction collector immediately because it has to pass through the tubing, the Conductivity Flow Cell, and the Diverter Valve first. The Delay is defined as the period in which the fluid is moving through the path from the UV Flow Cell to the Fraction Collector Drop Head.

When the Delay is set to a value greater than 0, each fraction advance is delayed by the value set after the event mark is drawn to allow the fluid in the UV Monitor Flow Cell to travel to the Fraction Collector Drop Head. In this way the fluid generating the UV Monitor signal at the time the event mark is drawn is actually the first fluid delivered to the corresponding fraction in the collector. When the Delay is set to 0, the event mark and the fraction advance occur simultaneously, and the first fluid collected in the fraction is not the fluid generating the UV Monitor signal.

Determining the correct Delay setting requires that you know the volume of the fluid path between the UV

Monitor Flow Cell and the Fraction Collector Drop Head. This is best determined empirically using a syringe. To do this, first make certain the tubing path is full of fluid. Connect an empty syringe to the tubing at the Fraction Collector Drop Head, disconnect the tubing from the UV Monitor Flow Cell, and then draw the fluid from the path into the syringe. Measure the volume of the fluid in the syringe; this is the Delay Volume.

The Delay setting is remembered between runs because it is assumed that the fluid path does not change between runs. Whenever the tubing is changed, the Delay setting should be redetermined unless the same diameter and length of tubing is used to make the connections.

Note: When running time-based methods, where delay time is used instead of delay volume, the correct delay setting must be determined using the formula below:

Delay Time = Delay Volume / Flow Rate

If multiple flow rates are used within the method, then calculate the delay using the flow rate when the most critical peaks are expected to elute.

5.4.2 Run in Progress

While a run is in progress, the Run Screen is displayed. Runs do not require that the operator be present; however there are a number of options available when present during the run. The information available during a run is described in section 5.4.2.1. There are three types of action which can be taken by the operator during a run: Holding a Run, Pausing a Run, and Manual Override. These are discussed in sections 5.4.2.2 though 5.4.2.4 respectively. The Chart Recorder trace is discussed in section 5.4.3.

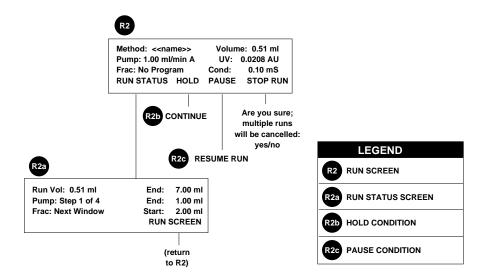


Figure 31. Run in Progress

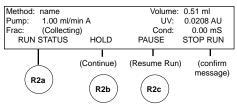
5.4.2.1 Information Available During a Run

There are two screens of information available ring a run which are described in Table 25.

Table 24.
Run Mode: Information Available During a Run

Reference Description

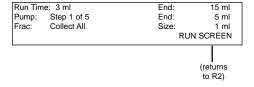




Run Screen: This displays the most important run information. The method name and the elapsed time or volume are shown on the top line of the LCD. The left side of the LCD shows the Pump and Fraction Collector status, and the right side of the LCD shows the UV Monitor and the Conductivity Monitor signals.

- Run Status: This displays the Run Status screen described below.
- Hold: This places the run on Hold. For a description of Holding a Run see section 5.4.2.2 below.
- Pause: This Pauses the run. For a description of Pausing a Run see section 5.4.2.3 below.
- Stop Run: This stops the run without finishing the programmed method. To prevent accidentally stopping a run, there is a Yes/No confirmation screen after pressing this softkey.





Run Status Screen: The elapsed time or volume in the run and the total time or volume of the run are shown on line 1 of the LCD. The current pump step, the total number of steps in the method, and the end time or volume for the current step are shown on line 2. The fraction collector status is shown on line 3. The information provided depends upon the fraction collection mode programmed in the method:

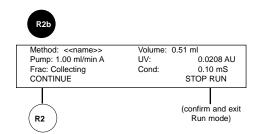
- Collect All: the fraction size
- Threshold: the threshold setting and whether the current UV Monitor signal is above or below the threshold
- Collection Windows and Threshold plus Collection Windows:
 - Inside a Window: the current window number, the total number of windows in the method, and the end time or volume for the current window
 - Outside All Windows: the next window number and when it will start

5.4.2.2 Holding a Run

Runs can be placed on Hold manually by pressing the Hold softkey on the Run Screen or they may occur automatically when an Alarm is programmed with Hold On. For information on programming Alarms with Hold On see section 5.3.6.

Table 25. Run Mode: Run Hold

Reference Description



During a Hold, all instruments continue to operate as they were when the Hold took effect; however the elapsed time or volume in the run stops changing. The pump continues to deliver the same buffer even if the Hold is placed in the middle of a gradient. For example if a 0-50%B gradient was placed on Hold at 33%B, then 33%B will continue to be delivered. If fractions were being collected, then they continue to be collected with the same fraction size and fraction advances. The **Continue** soft-key concludes the Hold by starting the elapsed time or volume counter.

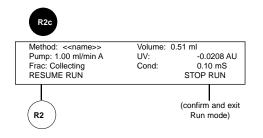
Note: Since the elapsed time or volume is not increasing during a Hold, the run will go on indefinitely. This can cause buffer containers to become empty, fraction collectors to run out of tubes, and other problems. This feature must be used carefully.

5.4.2.3 Pausing a Run

Runs can only be Paused by pressing the Pause softkey on the Run Screen they cannot be programmed to occur.

Table 26. Run Mode: Run Pause

Reference Description



During a Pause, all instruments stop functioning and the elapsed time or volume will not change. The **Resume Run** softkey concludes the Pause by starting all instruments and the elapsed time or volume counter.

During a Pause, the method itself can be changed before the run is resumed. This is accomplished by pressing the **Program** mode key to enter Program mode. The Run mode key light starts flashing as a reminder that a run in progress has been paused. While in program mode, the method can be edited normally with a single important exception. The exception is that the parts of the method which have already occurred cannot be changed. The pump step in progress can be edited, but doing so will split the step into two steps: the part of the step which has already occurred will be the first of the two steps, and the part of the step which has not yet occurred will be the second step. As a result of the split, the number of steps in the method will increase by one. After editing the method, a choice will be presented to either replace the original method, to save the modified method using a new name, or to finish the run with the modified method not saved. Completing this choice returns to the Run Paused screen.

For information describing how to edit the method, and the naming and saving options, see section 5.3 Programming Mode.

5.4.2.4 Manual Override

Many of the operations which the BioLogic LP instruments can perform in Manual mode can also be performed in Run mode. To access these capabilities, press the instrument which is appropriate for the desired action. The Run Mode Manual Override screens are almost identical to the Manual mode screens and function in the same way. For a description of how to use these screens, consult section 5.2 Manual Mode.

There are some consequences of using the manual override capabilities which must be understood. The list below describes the operations which are available, how to accomplish them, and any important information to know about using the capabilities.

- Event Marks: Using the Event Mark key to draw an event mark on the chart recorder trace will make an identical mark to the fraction advance marks and will make interpreting the chart recorder trace more difficult. See section 5.4.3 for additional information.
- Flow Rate: Changing the Flow Rate can be done using the Pump override screen. The new flow rate will take effect immediately and will last until the next pump step starts. When the next pump step starts, the programmed flow rate will be used. To make a permanent change to the flow rate, you must Pause and edit the method, see section 5.4.2.3.
- Fraction Size: Changing the Fraction Size can be done using the Collector override screen and the
 Frac Size softkey (the Edit softkey must be used when Thresholds are used). This change is permanent unless Collection Windows are in use. When Collection Windows are used, the new
 Fraction Size effects only the current or next window. During subsequent windows the fraction size
 will be as originally programmed within the method. Since using this capability changes the number
 of fractions collected, remember to consider how many tubes are available in the collector.
- Fraction Advance: The fraction collector can be advanced using the Collector override screen and the Advance softkey. A normal fraction advance (including an event mark and any Delay set) will occur. Since using this capability changes the number of fractions collected, remember to consider how many tubes are available in the collector.
 - Note: When the **Advance** softkey is pressed, it cannot be pressed again until the fraction advance actually occurs. This is only applicable and noticed when the Delay setting is greater than 0.
- Collect or Divert: The state of the Diverter Valve can be changed using the Collector override screen and the Collect or Divert softkey (which key is available depends upon the current state of the Diverter Valve). When using "Collect All" the override is permanent, however when using other collection modes the override is not permanent.
 - In "Threshold", "Collection Windows", or "Threshold plus Collection Windows" modes this override lasts until the Window or Threshold would have the opposite effect. For example, in Threshold mode, pressing Collect while below the threshold setting causes fractions to be collected until the UV signal passes above the threshold setting and then back below the setting again. In Threshold mode, pressing Divert while above the threshold setting causes fraction collection to stop until the UV signal passes below the threshold setting and then rises above it again. In Collection Windows mode, pressing Collect before a window starts causes fraction collection to start until the next window ends. In Collection Windows mode, pressing Divert while inside a window causes collection to stop until the next window starts. Although the situation is more complex in Threshold plus Collection Windows, the same rules are applicable. Since using this capability changes the number of fractions collected, remember to consider how many tubes are available in the collector.
- **UV Monitor**: The UV Monitor can be controlled exactly like it is in Manual mode, except that the lamp cannot be turned off.

- Conductivity Monitor: The Conductivity Monitor can be controlled exactly like it is in Manual mode, except that the Flow Cell cannot be calibrated.
- Chart Recorder: The Chart Recorder can be started and stopped at any time during a run.

Note: The Chart Recorder always starts when a run begins and always stops when a run ends.

There are some operations which are not allowed during a run. Manual override operation of the Alarms and the Valves are not allowed during a run. Changing the operation of these features can have large effects on the run, and to use these features the run must be Paused and edited. See section 5.4.2.3 for information on doing this.

Note: Although the Valves cannot be controlled by Manual Override, pressing the **Valve** instrument key displays a screen showing the state of each valve.

5.4.3 Interpreting the Chart Recorder Trace

The chart recorder trace contains the following information. Pen 1 is the UV Monitor signal and also contains the event marks. Pen 2 is the Conductivity Monitor signal. When a run is started there is a special large event mark indicating this. Each time the fraction collector is advanced there is a normal event mark. Each time the Event Mark key is pressed there is a normal event mark.

The BioLogic LP is designed to place an extra fraction advance between collection periods when the Diverter Valve sends the fluid to waste. The extra fraction is called the "Delay Tube". When the Delay is set to 0, this tube will be empty; but when the Delay is greater than 0, this tube will contain a volume equal to the delay setting. This design is used for two reasons which are described below.

The first reason for the Delay Tube is to insure that for each gap between event marks there is a corresponding fraction collected. When the fluid is diverted between two different collection periods, there is a gap between the event mark signaling the end of the first collection period and the event mark signaling the start of the second collection period. The Delay Tube is the tube "collected" during this time. Since this feature is present, you can count the gaps between event marks and they will correspond to the number of fractions collected. It is important to remember that pressing the **Event Mark** key will cause extra event marks to be drawn and makes counting fractions more difficult.

The second reason for the Delay Tube is only important when the Delay setting is actually used, meaning that the delay setting is not 0. Setting the appropriate delay prevents contamination of the first fraction collected after a period when fractions were not collected; i.e., there is a "gap" in collection. The potential for this contamination occurs because the fluid which was between the Diverter Valve and the Fraction Collector Drop Head is trapped until collection resumes, when it will be placed into the next fraction. When the appropriate delay is set, the Delay Tube collects this fluid instead of having it contaminate the first fraction.

6.0 MAINTENANCE AND TROUBLESHOOTING

The BioLogic LP System requires very little maintenance to assure reliable operation. This chapter discusses standard BioLogic LP components; discussion of optional components such as valves, is left to their separate documentation.

6.1 CLEANING AND STORAGE

During normal operation, spills and splashes may cause residues to form on component surfaces. To avoid damage or injury, unplug any instrument before cleaning it. 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's tray can be rinsed via the drain at the rear.

If the BioLogic LP will be stored for a long period, be sure to remove buffer salts from the valves and monitors by flushing with de-ionized water followed by a 20 % solution of ethanol to prevent microbial growth.

6.2 PUMP CALIBRATION

Pump calibration is important for a method programmed using volumes, because the BioLogic LP system uses the displayed flow rate to determine fraction size and for gradient control. The BioLogic LP calculates the flow rate by multiplying the pump head speed (rpm) by a flow rate factor determined during calibration.

The pump should be calibrated:

- 1. When the system is new. You must tell the system which size tubing is installed.
- 2. When changing tubing size.
- 3. When new tubing is installed or columns with different counterpressure are installed. For best accuracy, "user calibration" (described on the following page) is recommended.
- 4. Whenever the observed flow rate is reduced due to tubing wear, "user calibration" (described on the following page) is recommended. Worn tubing can be recognized by stretching, flat spots, or holes. If possible it is best to replace worn tubing.

The procedures for performing Nominal Calibration and User Calibration are discussed on the following page.

Note: For best flow rate accuracy, before calibrating your pump, check the adjustment of the platen pressure screw. (This is the large slot-head screw located on the pump head, near the lever that releases the platen.) First turn the platen adjustment screw counterclockwise until it stops. Then turn the screw clockwise according to the table below.

Number of Turns	
Tubing ID	From Fully Open
0.8 mm (1/32")	5
1.6 mm (1/16")	4
3.2 mm (1/8")	3

Also check the condition of the tubing; fatigued "flattened" tubing reduces the output of the pump.

6.2.1 Nominal Calibration

Nominal flow rate factors for the three standard tubing sizes (0.8, 1.6, and 3.2mm ID) are programmed into the system at the factory. Many users find that the flow rate accuracy obtained with "nominal" calibration is sufficient for their purposes. To use the nominal calibration values:

- 1. At the BioLogic LP's front panel, press the **Manual** mode button, followed by the **Pump** instrument button. This will display the pump's Manual mode screen.
- 2. Press the **Flow** softkey.
- 3. Press the **Calibrate** softkey. From the three tubing size options, press the softkey for the tubing size you will use.
- 4. Press the **Nominal** softkey. The system then will use the nominal calibration for the tubing size you have selected.

6.2.2 User Calibration

The user calibration feature allows the user to determine a flow rate factor experimentally and to program this factor into the system. This procedure is advisable when using columns with high counterpressure, when using pump head tubing of a non-standard size, or whenever precise flow rate accuracy is desired.

For best accuracy, calibrate the system with your column installed, and all other system components connected. Set the calibration flow rate to the flow rate you will be using during your run. If multiple flow rates will be used, calibrate at the flow rate for the most critical part of the separation.

To perform a user calibration,

- 1. Connect your system as you intend to use it, including the column. Place the inlet tubes in containers of buffer. Make sure the "waste" tube from the system also will reach a graduated cylinder placed on the bench.
- 2. Press the **Manual** mode button, followed by the Pump instrument button to display the Pump's main Manual mode screen.
- 3. With the "waste" tube in a waste container, press the Purge softkey to purge air from the system.
- 4. When the purge is complete, from the pump's main Manual mode screen, press the **Flow** softkey.
- 5. Press the **Calibrate** softkey to display the three tubing size options. Press the softkey for the tubing size you will be using. If you are using a non-standard tubing size in the pump head, press the **Other** softkey and proceed to step 7; the system will calibrate at 25% of full pump speed.
- 6. Press the **Set Flow** softkey, followed by the **Flow Rate** softkey. Using the numeric keypad, enter the flow rate for the calibration. Press the **OK** softkey.
- 7. Press the **Time** softkey. Using the numeric keypad, enter the total time period for the calibration. (For best results, enter a time of 5 minutes.) Press the **OK** softkey.
- 8. Place the "waste" outlet tube into the empty graduated cylinder. Press the **Start** softkey. The pump will run and the display will count down the time remaining.
- At the end of the calibration period, the pump will stop. Using the numeric keypad, enter the total
 volume delivered to the graduated cylinder. Press the **OK** softkey. The system is now calibrated for
 the tubing and system configuration used.

6.3 FLUSHING/CLEANING VALVES, FLOW CELLS, AND FILTERS

6.3.1 Rinsing Valves and Flow Cells

When the BioLogic LP will not be used for more than a day or two, it is important to flush salt solutions from the valves and flow cells. Over time, salt solutions may crystallize inside of valves and flow cells, causing damage. To flush the system:

- 1. Remove the column from the system and connect the column inlet tube to its outlet tube.
- 2. Place all buffer inlet tubes in water, and from the Pump's Manual mode, select the Purge softkey. This runs the pump at maximum speed.

Note: If the system will be stored for more than a week, follow the water wash with a 20% ethanol to the water to retard microbial growth in the system.

- 3. As the pump runs, use the Pump's Manual mode to select buffers "A" through "E" in turn, allowing time for each inlet tube to clear.
- 4. Switch to Manual mode operation of the Valves. From the Valves manual mode screen, select the Divert softkey. Switch the diverter valve from "Collect" to "Divert" to Waste. Allow water to flow for 15 seconds, then switch back to Collect. If an SV-3 Column Bypass valve is installed, select the Bypass softkey from the Valves manual mode. Switch to "Bypass" mode for 15 seconds, then return to "Column" mode.

Note: If you choose to write a Method to accomplish the procedure above, keep in mind that you will need to manually switch the SV-3 Bypass valve.

6.3.2 Cleaning Valves and Flow Cells

If valves and/or flow cells show signs of clogging, they can be cleaned as described below. Cleaning times may be extended for severe clogging or microbial growth.

- 1. Remove the column from the system and connect the column inlet tube to its outlet tube.
- 2. Pump 1 M NaOH (Sodium Hydroxide) through the system at 1 ml/min, for 30 minutes. If valves are being cleaned, manually switch valve positions several times during this procedure.
- 3. Flush the system with water. If valves are being cleaned, manually switch valve positions several times during this procedure.
- 4. Pump 1 M HCI (Hydrochloric acid) through the system at 1 ml/min, for 30 minutes. If valves are being cleaned, manually switch valve positions several times during this procedure.
- 5. Flush the system with water as described in Section 6.3.1 above.

6.3.3 Cleaning the UV Optics Module's Filters

The 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.

6.4 CARE OF THE PROPORTIONING VALVE AND MIXER

Normally, the only maintenance required is to flush the Proportioning Valve with water. To do this, use the Pump's manual mode to set the buffer to 50%B. Place both inlet tubes in a vessel of water and pump until the salt is purged from the valve. Do not leave high salt buffers in the valve, as the crystallized salt may damage the valve. To more vigorously clean the mixing chamber of residue such as salt:

- 1. Disconnect the Mixer's cable from the BioLogic LP Controller.
- 2. Use a 3/8" socket driver or box wrench to carefully remove the top of the mixer by rotating the top counterclockwise.
- 3. The magnetic stir bar may be removed from the mixing chamber by turning the unit upside down and tapping the unit in the palm of your hand until the stir bar drops out of the chamber.
- 4. The mixing chamber and top may now be cleaned as required.
- 5. The chamber can be flushed by connecting a luer-tipped syringe to the "A" port and slowly passing fluid through the valve and mixing chamber. Do **not** attempt to force liquid through the closed "B" port; this may damage the proportioning valve.

To reassemble the unit:

- 1. Wipe the mixing chamber and threads clean.
- 2. Insert the stir bar back into the mixing chamber with the flat end of the stir bar toward the bottom of the chamber. Gently push the stir bar to the bottom of the chamber.
- 3. Wipe the top and threads clean.
- 4. If the 1.0cm O-ring seal on the top is damaged it must be replaced or the mixer will leak. (Bio-Rad part number for O-ring: 910-0075.)
- 5. Use a 3/8" socket driver or box wrench to screw the top on the mixer by rotating the top clockwise until the lip around the top is just flush with the top of the mixing chamber. The seal is made by a properly seated O-ring, not by the screw threads. To avoid damaging the unit, do not overtighten the top.

6.5 REPLACING THE LAMP IN THE UV OPTICS MODULE

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.

Note: Before performing the following procedure, make sure the BioLogic LP Controller is turned off. This will eliminate the risk of electric shock to you or damage to the Optics module.

To remove the lamp from the UV Optics Module,

- 1. Turn off all electrical power to the BioLogic LP Controller and unplug the UV Lamp cable. Wait 10 minutes for the UV Optics Module to cool.
- 2. Remove the filter tray and flow cell, as discussed in the previous section.

- 3. Remove the screws holding the optics module together and remove the bottom half of the case.
- 4. Pull the UV lamp out of its holder and unplug it from its connector.

When inserting the new UV lamp, **never** handle the quartz surface of the lamp; grease and fingerprints will damage the lamp. This procedure is also discussed in the instruction sheet for the replacement lamp.

Note: When re-assembling the case, be sure the O-ring is properly seated. An incorrectly seated O-ring will cause the unit to leak light and give poor performance.

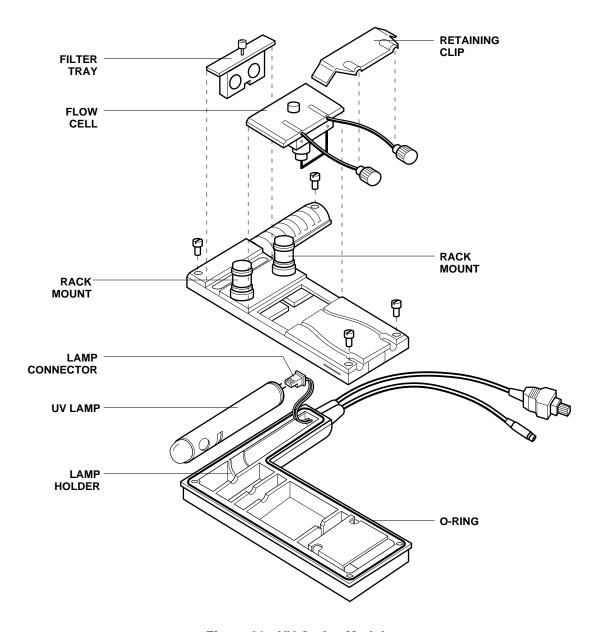


Figure 32. UV Optics Module

6.6 TROUBLESHOOTING

Listed below are some guidelines for troubleshooting the system:

Unstable baseline. This may be caused by any of the following:

- Bubbles in Flow Cell: BioLogic LP Flow Cells are designed to clear bubbles. Confirm that the flow
 cell tubes are connected properly. Note the flow direction arrows on top of the UV Optics module
 case. Be sure that the UV Optics module is mounted upright on the rack, with the mounting posts
 pointing down.
- Dirty or Obstructed flow cell: Turn the UV lamp "off", remove the flow cell, and look through the sample window. If visible obstruction is seen, clean the flow cell (see section 6.3).
- Light leaks: The optics module case is sealed against light by three O-rings- One each on the flow
 cell and filter drawer, and one between the case halves. To check for light leaks, pump buffer
 through the unit, and squeeze the optics module case halves together gently, using your thumb and
 forefinger. Observe the chart recorder pen- if squeezing the optics module causes noticeable deflection of the UV (channel 1) pen, a light leak is probable.
 - If a light leak is suspected, turn the UV lamp off, remove the flow cell and filter drawer, and confirm that the O-rings are in place. Remove the four screws holding the case halves together, separate the case halves, and confirm that the sealing O-ring is in place. Reassemble the optics module and repeat the test for light leaks.
- Lamp exhausted: If the UV lamp is nearing the end of its service life, the base line may become unstable. Replace lamp.

Flow rate is not as displayed.

- Check for worn tubing, and confirm that platen pressure is adjusted properly. Refer to section 4.2.
- Check pump calibration:
 - a. If Nominal calibration is used, confirm that proper tubing size is selected. Consider performing User calibration. Refer to section 6.2, Pump Plumbing and Calibration.
 - b. If User calibration is used, repeat calibration- it is best to calibrate with column, etc. installed in system, and with the actual buffers used for the separation. Set the flow rate to the same rate used for the separation. Refer to section 6.2, Pump Plumbing and Calibration.
- Check for obstructed tubing, columns, or valves.

Gradient not as expected.

- 1. Check that the buffers are prepared properly, and the correct method has been selected.
- 2. Check the range setting on Gradient Monitor.
- 3. Check switch settings and connections of Chart Recorder. All switches should be set to position labeled in green, and the conductivity signal cable (banana plugs) must be plugged into channel 2.
- 4. Measure actual flow rate through buffer "A" port with stopwatch and graduated cylinder, and compare to actual flow rate. If the difference between indicated and actual flow rates is significant:
 - a. Check for worn tubing and improper platen adjustment;
 - b. Check for obstructed tubing, fittings, columns, or valves. You may wish to clean the system as described in section 6.3, Flushing/Cleaning Valves, Flow Cells, and Filters.
- 5. Measure actual flow rate through buffer "B" port with stopwatch and graduated cylinder, and compare to actual flow rate through "A" port. If the actual flow rates differ significantly, check for obstructions in the proportioning valve or in the tubing leading to the valve.

Error Messages.

Under certain abnormal circumstances, the BioLogic LP may display an error message. The format of the error message is:

BioLogic LP Error Trap! <Text> <Text> Cycle system power to restart.

If an error message is seen, turn power "off", wait 15 seconds, then turn power "on". Turning the power off will not lose methods or calibration values. If the message reappears, write down the exact text displayed, and then contact your local Bio-Rad representative. (In the U.S., call 1-800-4-BIORAD for technical assistance.)

APPENDIX A. SPECIFICATIONS

BioLogic LP System

Power 115 V ~ 5.3 A

230 V ~ 3.5 A 50 - 60 Hz

Operating temperature 2° to 40°C, ≤95% humidity

Construction Material Polypropylene and other solvent resistant plastics

Flow rate range (per channel)

0.05 to 20 ml/min (depending on tubing diameter)

Pump head speed 25 rpm (max.)

Tubing diameter 0.4 mm (ID) to 3.2 mm (ID), maximum 1 mm wall thickness

Speed adjustment .01 ml/min

Speed stability 1% full scale

Counterpressure (max.) 30 psi (2 kg/cm2 or bars)

UV Monitor

Mercury/Phosphor Lamp

Wavelength: 280 and 254 nm (both filters supplied)

Auto Zero Feature

Eleven fixed ranges, 2.0 to 0.001 AU Full Scale User set range 2.0 to 0.001 AU Full Scale

Cuvette volume: 80 µl Path length: 2 mm Illuminated volume: 3 µl

Full Scale output to chart recorder: 1 volt Non Scaled Integrator output: 1 volt @ 2 AU

Conductivity Monitor

AC excitation

Nine fixed ranges, 500 to 0.5 mS/cm Full Scale

User Set minimum and maximum values from 500 to 0.5 mS/cm

Flow Cell swept volume: 8 µl Accuracy: Within 2% of full scale

Full Scale output to chart recorder: 1 volt

Proportioning Accuracy: Within 1 % of full scale (3% to 97% B)

Gradient Linearity: Within 3 % of full scale (3% to 97% B)

Valve Control

- Proportioning valve/Mixer module, Diverter valve (SV-3), Bypass valve (SV-3), and Buffer Select valve (SV-5, or SV-3)
- One low-pressure manual valve (MV-6) for sample injection

Fraction Collection

- Model 2128 Fraction Collector (collection by Collect All, Threshold, Collection Windows, Collection Window + Threshold)
- Model 2110 fraction collector (collection by Collect All; collection by Threshold, Collection Windows, Collection Window + Threshold available with SV-3 Diverter valve)

Chart Recorder Control

Model 1327 Dual pen recorder. Paper feed Start/Stop, Pen Up/Down

APPENDIX B. WARRANTY AND ORDERING INFORMATION

The BioLogic LP System is warranted for 1 year against defects in materials and workmanship. If any defects should occur during this warranty period, Bio-Rad Laboratories will replace the defective parts without charge. 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 and fittings.

For additional help, contact your local Bio-Rad representative. In the United States, call Technical Service at 1-800-4BIORAD.

WARRANTY INFORMATION

Model:	
Warranty Period:	

ORDERING INFORMATION

	BioLogic LP Systems
731-8300	BioLogic LP, 110V LP Controller, System Rack, UV Optics Module, Conductivity flow cell, Injection valve, Proportioning Valve/Mixer, tubing and fittings kit.
731-8301	BioLogic LP, 220V Same as 731-8300, except 220 V.
731-8302	BioLogic LP, with Model 2110 Fraction Collector, 110V LP Controller, System Rack, UV Optics Module, Conductivity flow cell, Injection valve, Model 2110 Fraction Collector, SV-3 Diverter Valve and System Cable 1, Proportioning Valve/Mixer, tubing and fittings kit.
731-8303	BioLogic LP, with Model 2110 Fraction Collector, 220V Same as 731-8302, except 220 V.
731-8304	BioLogic LP, with Model 2128 Fraction Collector, 110V LP Controller, System Rack, UV Optics Module, Conductivity flow cell, Injection valve, Model 2128 Fraction Collector, Model 2128 Diverter Valve and System Cables 3 and 15, Proportioning Valve/Mixer, tubing and fittings kit.
731-8305	BioLogic LP, with Model 2128 Fraction Collector, 220V Same as 731-8304, except 220 V.
750-0251	BioLogic Rack
750-0268 750-0260 750-0261 750-0262 750-0263 750-0264 750-0265 750-0266 750-0269	Rack expansion kit (2 trays, 2 vertical bars, 16 sleeves) Column clamp set Rack tray, 1 (+8 sleeves, 1 drain/plug) Vertical bar, long, 2 Vertical bar, short Horizontal bar kit (2 tie bars, 4 bar clamps) Bar clamps, 5 Cable Manager clips, 4 System wrench set

Ordering Information (Continued)

	Peripherals
731-8122 731-8120	Model 2110 Fraction Collector, 110 V Model 2110 Fraction Collector, 220 V
731-8123 731-8124	Model 2128 Fraction Collector, 110 V Model 2128 Fraction Collector, 220 V
731-8250	Model 1327 Econo Recorder, with USA, Canada, Japan, Mexico, Taiwan, and Latin America power adapter
731-8253	Model 1327 Econo Recorder, with UK, Commonwealth power adapter
731-8254	Model 1327 Econo Recorder, with Australia and New Zealand power adapter
731-8255	Model 1327 Econo Recorder, with European power adapter
	Valves
731-8320 731-8321 731-8322	BioLogic LP Injection Valve (MV-6) BioLogic LP Buffer Select Valve BioLogic LP Column Bypass/Fraction Collector Diverter Valve
731-8323	BioLogic LP Proportioning Valve/Mixer Module
731-8324 731-8165 731-8166 731-8167	BioLogic LP UV Optics Module Flow Cell, replacement Lamp , replacement Filter assembly, 280 and 254 nm
731-8155	Conductivity Flow Cell replacement
	Cabling
731-8261	System Cable 1, 8-pin mini-DIN to DB-9 connector, to connect a Model 2110 Fraction Collector to the BioLogic LP
731-8262	System Cable 2, 8-pin mini-DIN to 8-pin standard DIN to connect a Model 1327 Chart Recorder to the BioLogic LP
731-8263	System Cable 3, 8-pin mini-DIN to 8-pin mini-DIN to connect a Model 2128 Fraction Collector to the BioLogic LP
731-8264	System Cable 4, 8-pin mini-DIN to banana plug cable, connects the Model 1327 Chart Recorder to the BioLogic LP Conductivity Chart output.

Ordering Information (Continued)

	Cabling (continued)
731-8267	System Cable 7, 8-pin mini-DIN to bare wires, connects the BioLogic LP to non-Bio-Rad components
731-8269	System Cable 9, 8-pin mini-DIN to Pharmacia FRAC-100, to connect a Pharmacia FRAC-100 fraction collector to the BioLogic LP
731-8283	System Cable 12, 8-pin mini-DIN to Isco DB-15 connector, to connect an Isco Retriever II collector to the BioLogic LP
731-8285	System Cable 14, 8-pin mini-DIN to Gilson connector, to connect a Gilson FC 203 fraction collector to the BioLogic LP
731-8286	System Cable 15, 15-pin D to mini-DIN to connect a Model 2128 Fraction Collector to the BioLogic LP.
	BioLogic LP System Fittings
731-8220	System Fittings Kit; 250 pieces
731-8221 731-8222 731-8223	Female luer with barb for 0.8 mm ID Tubing (25) Female luer with barb for 1.6 mm ID Tubing (25) Female luer with barb for 3.2 mm ID Tubing (25)
731-8224 731-8225 731-8226	Male luer with barb for 0.8 mm ID Tubing (25) Male luer with barb for 1.6 mm ID Tubing (25) Male luer with barb for 3.2 mm ID Tubing (25)
731-8228 731-8230 731-8232 731-8233	Female-to-Female luer (10) Male-to-Male luer (10) Female luer plugs (25) Male luer plugs (25)
731-8102 731-8103 731-8107	2-way stopcock, Female-to-Male luer; polycarbonate (10) 3-way stopcock, 2 Female to 1 Male luer; polycarbonate (10) 3-way stopcock; nylon, solvent resistant (10)
732-8300 732-8302	0.8 mm Barb-to-Barb connector (25) 0.8 mm Barb T-connector (25)

Ordering Information (Continued)

BioLogic LP System Tubing

731-8210 731-8211 731-8212	Silicone, 0.8 mm ID, 0.8 mm wall, 10 m length Silicone, 1.6 mm ID, 0.8 mm wall, 10 m length Silicone, 3.2 mm ID, 0.8 mm wall, 10 m length
731-8214 731-8215	Tygon, 0.8 mm ID, 0.8 mm wall, 10 m length Tygon, 1.6 mm ID, 0.8 mm wall, 10 m length
731-8207 731-8208 731-8209	PharMed, 0.8 mm ID, 1.0 mm wall, 10 m length PharMed, 1.6 mm ID, 1.0 mm wall, 10 m length PharMed, 3.2 mm ID, 1.0 mm wall, 10 m length
731-8240 731-8241 731-8242	Pump Tubing kit, 0.8 mm ID silicone, 20 precut lengths and 4 sets of fittings Pump Tubing kit, 1.6 mm ID silicone, 20 precut lengths and 4 sets of fittings Pump Tubing kit, 3.2 mm ID silicone, 20 precut lengths and 4 sets of fittings
731-8247 731-8248 731-8249	Pump Tubing kit, 0.8 mm ID PharMed, 20 precut lengths and 4 sets of fittings Pump Tubing kit, 1.6 mm ID PharMed, 20 precut lengths and 4 sets of fittings Pump Tubing kit, 3.2 mm ID PharMed, 20 precut lengths and 4 sets of fittings



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