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# Micro Analytical Gradient Integrated Chromatograph 2002

# MAGIC 2002™
# Operator's Manual

# Version 2.0

**Part No. 602-00000-00**
**01/99**

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# WARRANTY & SERVICE AGREEMENT

## MAGIC ™ Warranty Policy

Michrom BioResources, Inc. (the "Company") warrants the Micro Analytical Gradient Integrated Chromatograph 2002 ™ (MAGIC 2002™ ) for a period of one year from the date of delivery by the Company to the original buyer against defects in materials and workmanship under normal use and maintenance, as described in the operating instructions within this manual. This warranty covers all of the MAGIC 2002™ components, excluding expendable items such as columns, check valves, pistons, piston seals, lamps, trap cartridges, rotors, purge valves, bottles and tubing. The exclusive remedy for defects in material and workmanship, at the option of Michrom BioResources, Inc., shall be replacing or repairing the parts determined to be defective.

This warranty is void in the event the MAGIC 2002 ™ is altered or modified other than by or at the direction of Michrom BioResources, Inc.

The foregoing warranty is exclusive and all other warranties, whether expressed or implied, including any warranties of merchantability and warranties of fitness for purpose, but without limitation thereto, are expressly excluded. Under no circumstances shall the company be liable for any consequential damages or any indirect or incidental damages.

## Service Agreements

After the expiration of the warranty period, extended service agreements are available for purchase through Michrom BioResources, Inc. Please contact Michrom BioResources, Inc. for detailed information on current service agreement options.

# SAFETY INFORMATION

This manual contains warnings and precautionary statements that can prevent personal injury, instrument damage, and loss of data if properly followed.

## Specific Hazards

Every instrument has specific hazards associated with it. Reading and complying with the following precaution instructions will help ensure the instrument's safe long-term use and the operator's personal safety.

1. Before supplying power to the MAGIC 2002™ HPLC (High Performance Liquid Chromatograph), check to ensure that the voltage and fuses are set appropriately for your local power supply. Never run the instrument at more than 8% below the nominal line voltage!

2. The power cord must be inserted into a power outlet with a protective earth contact (ground). When using an extension cord, make sure that it is also grounded.

3. Do not change the external or internal grounding connections. Tampering with or disconnecting these connections could endanger you and/or damage the instrument.

4. Never operate the instrument with the top cover removed except during troubleshooting procedures under the direction of a Michrom BioResources, Inc. service representative.

5. Do not turn the instrument on if you suspect that it has incurred electrical damage. Disconnect the power cord and contact Michrom BioResources, Inc. or its authorized representative in your area.

6. Damage can result if the instrument is stored for prolonged periods of time under unfavorable conditions (*e.g.*, temperature extremes, humidity, etc.). Prior to prolonged storage or shipment, the instrument should be thoroughly flushed with wash solvent (40/40/20 Acetonitrile/n-propanol/$H_2O$) to remove salts, buffers, acids, bases or other chemicals that could cause corrosion or precipitation in the system.

7. Always disconnect the power cord before attempting any type of electronic maintenance.

8. Capacitors inside the instrument may still be charged even if the instrument is turned off. Proper precautions should be taken when working with electronic components in the instrument.

9. Never try to repair or replace an instrument component not described in this manual without first consulting Michrom BioResources, Inc., or its authorized representative in your area.

## Good Laboratory Practices

- **Maintaining Records** Michrom BioResources, Inc. recommends that records of system operating conditions be maintained. At a minimum, keep a chromatogram of a standard test mixture along with the associated system conditions. Careful comparison of retention times, peak shapes, column pressure, peak sensitivity, and baseline noise can provide valuable clues to identifying and solving problems.

- **Chemical Toxicity** The large volume of toxic and flammable solvents used and stored in laboratories can be potentially dangerous. In addition, the potential hazards posed by samples should be recognized. Special care should be taken to follow all precautions necessary to ensure proper ventilation, storage, handling, and disposal of both solvents and samples. Become familiar with the toxicity data and potential hazards associated with all chemicals by referring to the manufacturers' Material Safety Data Sheets (MSDS).

- **Sample Preparation** Filter samples that might contain particulate matter using a 0.45µm filter. Always consider the solubility of your sample in the mobile phase. Sample precipitation can plug the system by obstructing the flow through the injector and/or column. This obstruction may result in damage to parts of the system. If possible, samples should be dissolved in the starting mobile phase (Solvent A) or a solvent of lower chromatographic mobility than the mobile phase.

- **Solvent Requirements** Many chemical manufacturers provide high-purity or spectro-quality reagents that are free of chemical impurities. Typically, HPLC-grade solvents do not require filtration. Choose a mobile phase that is compatible with the sample and a column that is specific for your separation requirements. The structural materials that comprise the solvent fluid pathway in the MAGIC 2002$^{TM}$ include stainless steel, ceramic, sapphire, quartz and PEEK. Caution should be taken when using strong acids, strong bases, non-volatile buffers, THF (Tetrahydrofuran) and Chlorinated solvents.

- **Degassing the Solvents** Degas the solvents as instructed under the Basic Operation section in this manual (Chapter 2; Section I.).

- **Solvent Disposal** All solvent waste lines on the MAGIC 2002 $^{TM}$ terminate in the solvent waste container in the waste compartment on the right side of the instrument.

Empty the waste container on a regular basis. Many solvents have special disposal requirements. Follow all governmental regulations when disposing of any chemical.

- **High-pressure Systems and Leaks** Little danger arises from the pressure in an HPLC system. However, if a leak does occur, it should be corrected as soon as possible in order to maintain system integrity and operator safety. It is recommended that eye and skin protection be utilized as standard laboratory attire when working on an HPLC system and that the system be shut down and returned to atmospheric pressure before attempting maintenance.

# SYSTEM REQUIREMENTS

**Power:** 100-240 VAC/47-63 hz/600Va

**Gas:** Helium, 20-200 PSI, high pressure input

**System control:** Windows 95 (or NT 4.0) Full Digital Control

**Location:** Ventilation slots (air inlet and air outlet) for the column forced-air compartment require at least 2 inches of clearance

The air inlet to the instrument is located in the front of the waste/wash bottle compartment (*See Figure 4, page 14)*

The air outlet (cabinet fan) from the instrument is located on the top rear panel (*See Figure 3, page 13)*

**Environmental:** Temperature 20-25 degrees Celsius
Humidity 20-90%

**Physical:** Size: 19”w x 26”h x 23”d
Weight: 140 lbs.

**PC Requirements:** CPU: Pentium 166 MHz minimum
Memory: (Minimum Requirements)

| | NT | Win95 |
|---|---|---|
| Clients | 32MB | 24MB |
| EZServers | 64MB | N/A |

Network: Microsoft networking (TCP/IP req. for client server)
O/S: Win95 service pack 1 (DCOM95 1.1 req. for client server)
WinNT 4.0 service pack 3
Disk: At least 80 MB free disk space
Note: Service packs and DCOM95 are available on the Elite 2.1 CD-ROM
***Note: Do not use Open GL Screen Savers with EZChrom Elite.***

**Controller Kit:** MAGIC 2002™ HPLC Control System
OR
MAGIC 2002™ HPLC Control/Data System

**Flow Range Kit:** Analytical Flow Range Kit
OR
Microbore Flow Range Kit
OR
Capillary Flow Range Kit

# LIST OF SPARE PARTS AND CONSUMMABLES

**SPARE PARTS**

| Part Number | Description |
|---|---|
| 004-32012-01 | O-ring, Viton |
| 004-61027-00 | Bottle, 1000mL, Plastic coated |
| 004-32033-00 | Bottle, Nalgene 2L |
| 003-51003/00 | Fuse, GMA, 5x20, 5A, 250VAC |
| 003-51004/00 | Fuse, GMA, 5x20, 3.5A, 250VAC |
| 003-51005/00 | Fuse, GMA, 5x20, 2.5A, 250VAC |
| 003-51006/00 | Fuse, GMA, 5x20, 2A, 250VAC |

**CONSUMMABLES**

| Part Number | Description |
|---|---|
| 602-25011-99 | Pump head Rebuild |
| 602-25027-88 | Replacement Check Valve, Inlet |
| 602-25028-88 | Replacement Check Valve, Outlet |
| 200-39000-00 | Replacement Deuterium Lamp |
| 004-25106-00 | 2u Titanium Filer Assy |
| 004-25131-88 | Repair Kit, Helium Check valve Assembly |
| 004-25003-00 | Upchurch 1/16 SS Male Nut |
| 004-25004-00 | Upchurch 1/16 SS Ferrule |
| 004-25005-00 | Valco 1/16 SS Male Nut |
| 004-25006-00 | Valco 1/16 SS Ferrule |
| 004-25027-00 | Union, ZDV, Peek, 10-32 |
| 004-65023-00 | Upchurch 10-32 Delrin Plug |
| 004-25094-00 | Optimize 10-32 Delrin Plug |
| 004-25008-00 | Upchurch Lite-Touch 10-32 Nut |
| 004-25009-00 | Upchurch Lite-Touch Ferrule, Front and Rear |
| 004-32001-00 | Tubing, Peek, .007” |
| 004-32002-00 | Tubing, Peek, .010” |
| 004-32004-00 | Tubing, Peek, .030“ |

# CHAPTER 1

# INSTRUMENT DESCRIPTION

## I. Overview

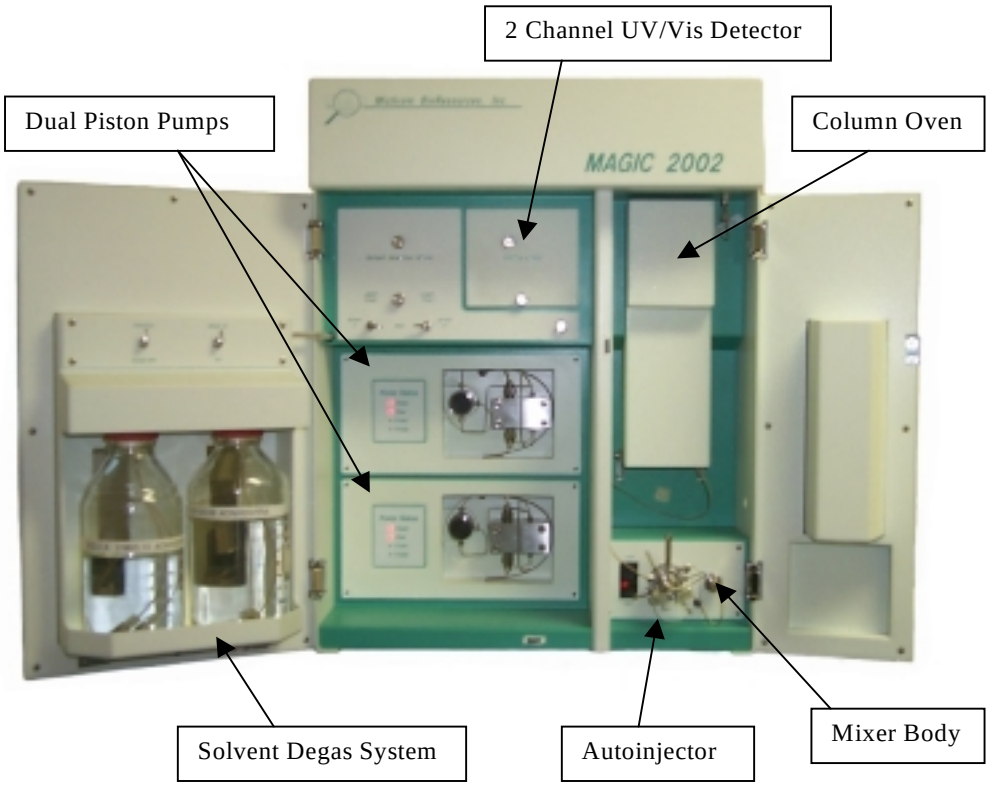
The MAGIC 2002™ is the first of a new generation of HPLC systems designed to let the user translate methods from analytical to microbore levels with no impact on the separation. The MAGIC 2002™'s dual pump high pressure binary gradient solvent delivery system will deliver accurate gradient flows from 0.5µl/min to 5.0 ml/min. The system will accept a wide range of column sizes from 50µm to 10mm ID and from 50mm to 250mm in length. Conventional analytical columns or Michrom BioResources, Inc.'s custom microbore columns or capillary columns can be utilized. The built-in dual wavelength UV/Vis detector has flow cells to cover the entire gradient flow range. Auxiliary detectors, including fluorescence, electrochemical, radioactivity or mass spectrometer can be added with no impact on extra column band broadening. The MAGIC 2002™ design allows for the connection of peripheral devices and accessory items that automate sampling, fraction collection and online sample preparation.

*Figure 1 MAGIC 2002™ with PC*

# II. Basic Components

The MAGIC 2002™ is a fully integrated HPLC system optimized for speed, sensitivity, recovery, and capacity without compromising reproducibility or resolution.

*Figure 2 MAGIC 2002™ with doors open*




## FEATURES

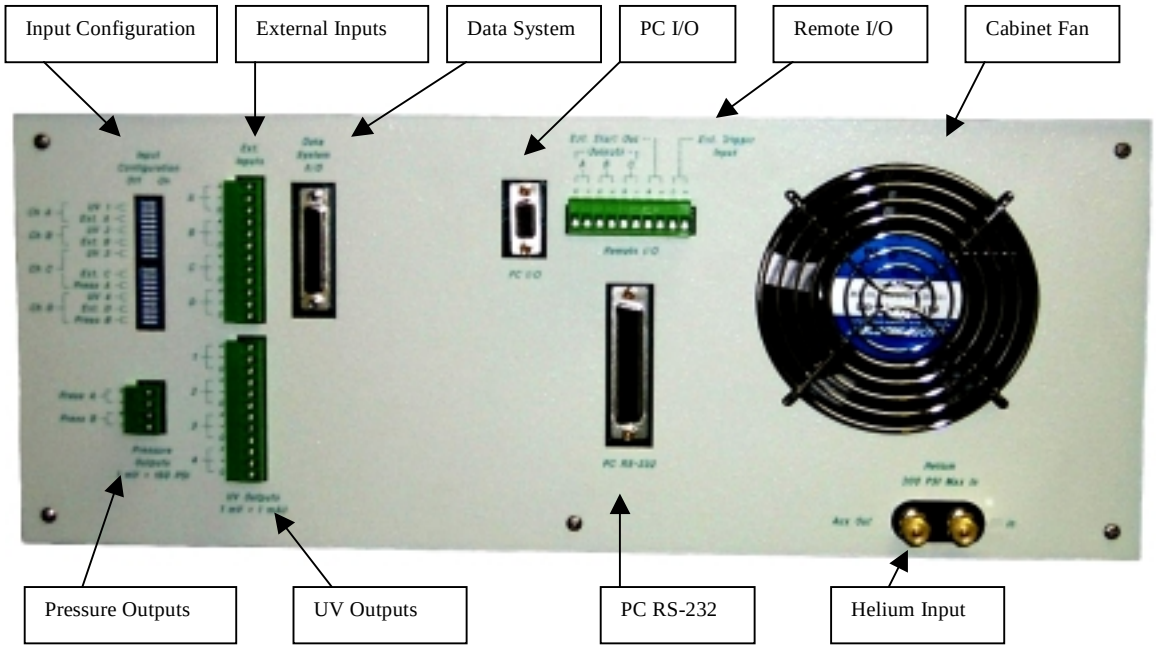
**Dual Range Piston Pumps** Micro flow or analytical pump heads deliver degassed solvents at precise flows down to 1% of total flow.

**Unique Flow Dynamics** Thorough mixing, rapid flush out, optimized post-column collection with minimum volumes.

**Custom Columns** Michrom BioResources, Inc. custom columns thread directly into micro or capillary flow cells, eliminating extra column volume; conventional columns can also be used with a standard column adaptor (Microbore P/N 602-25026-01; Analytical P/N 602-25026-02).

## DESCRIPTION OF INSTRUMENT REAR PANEL

*Figure 3 Rear Panel*




**Input Configuration** A series of switches for On/Off control of the Data System inputs.

**External Inputs** The location for the input of external signals to the data acquisition system (i.e. auxiliary detectors). The input configuration switch for the external input must be placed in the "on" position for the data system to accept an external input signal.

**Data System** The connection between the Data system PC board and the MAGIC 2002™ 25-pin A/D connector.

**PC I/O** The connection between the Data System PC board (or the I/O PC board) and the MAGIC 2002™ 15-pin I/O connector.

**Remote I/O** Remote I/Os that are programmable in a Procedure (A, B, C) (i.e., Fraction Collector start), and external input and output for auto sampler interfacing.

**PC RS-232** Connection between the PC RS-232 board and the MAGIC 2002™ 78-pin.

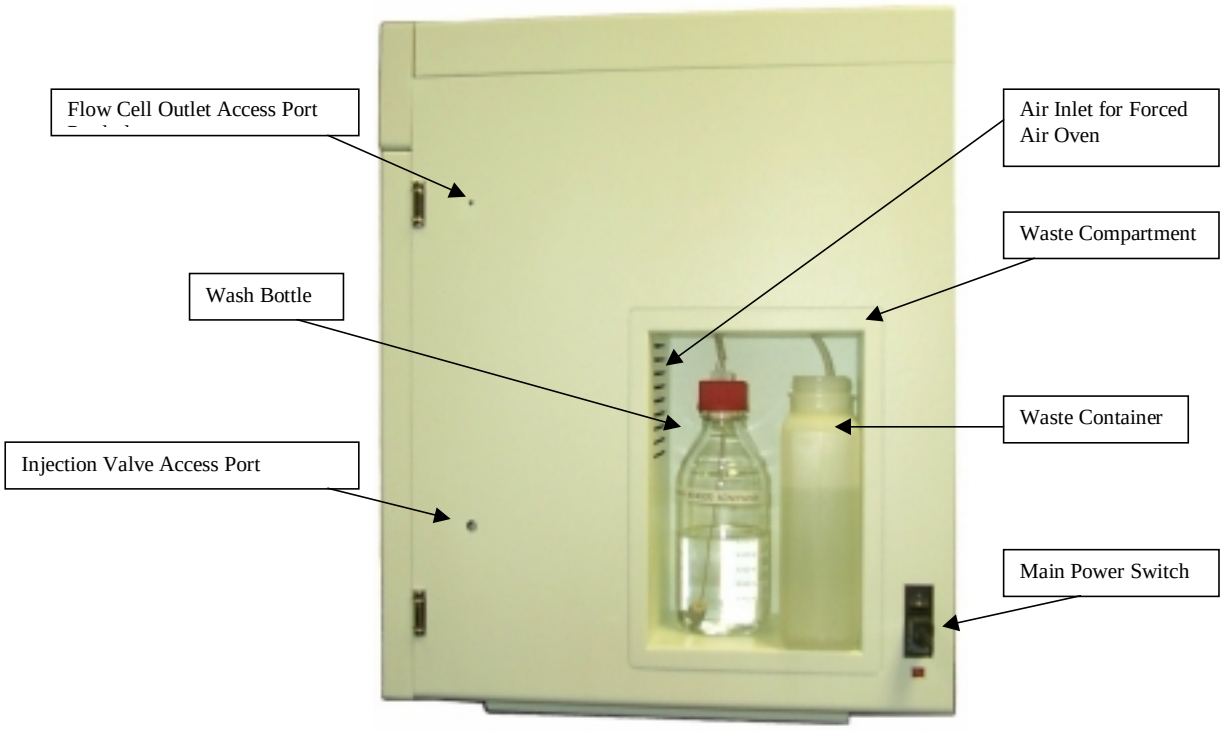
**UV Outputs** The connections between MAGIC 2002™ and external devices to collect UV data.

**Pressure Outputs** The connections between the MAGIC 2002™ and external devices to collect pressure information.

**Helium Connections** The location for connection of Helium to the MAGIC 2002™. The recommended input is 20-200 PSI.

## DESCRIPTION OF RIGHT SIDE PANEL

*Figure 4 Right Side Panel*




**Waste/Wash Compartment** Compartment designed to house the waste container and wash solvent bottle.

**Waste Container** 2-liter Nalgene bottle (***Note: All Solvent waste lines should go into the waste bottle and the bottle should be emptied when the waste solvent reaches the waste lines. If not, waste solvent may back flow out to the manual injection port.)***

**Wash Solvent Bottle** 1-liter solvent bottle for wash solvent (40/40/20 ACN/ProH/$H_20$).

**Main Power Switch** Contains power entrance block, main fuses, power switch and voltage select switch.

**Flow Cell Outlet Access Port** Provides accessibility to flow cell outlet for flow measurements or connection to peripheral devices (mass spectrometer, fraction collector, etc.)

**Injection Valve Access Port** Provides accessibility to injection valve for auto sampler or other in-line devices.

# III. Hardware Description

This section provides the user with a better understanding of the MAGIC 2002™ HPLC system and its components.

### Solvent Degas/Pressurization System

The Helium input line is located on the rear instrument panel (*Refer to Figure 3, page 13*). There is an additional Helium output for attachment of accessory devices such as the Michrom BioResources, Inc. Pulseless Flow Module. The solvent degas system internally regulates pressure to 6 PSI. The system gas is split to independently degas and pressurize the A/B solvent bottles, and to pressurize the wash solvent bottle.

### Solvent Bottle Degas/Pressurization System

There are two switching valves above the solvent bottles on the inside front door of the pump compartment (*Refer to Figure 5, page 16*). The first valve is a Helium shutoff valve and is used to introduce or interrupt Helium flow to the A/B solvent bottles. ***Please note that this shutoff valve does not stop Helium flow to the wash solvent bottle***. The second valve is the Pressurize/Sparge valve. In the sparge position, this valve allows Helium to freely enter the solvents and remove Oxygen (degas solvents). Excess air and Helium are vented from the solvents and bottles. When the Pressurize/Sparge valve is in the pressurize position, the bottles are sealed and placed under 6 PSI of head pressure on the solvents. ***Prior to opening a bottle to replace solvent, the Helium shutoff valve must be closed and the Pressurize/Sparge valve turned to the Sparge position. This action allows the bottles to vent and releases system pressure.***

The Helium to the solvent bottles has two functions. First, it serves to degas solvents by removing air that can cause problems with pumping, separation and detection in the HPLC. Second, it equalizes the pressure on the two solvents. This pressure equalization provides the stability required for the reproducible solvent delivery system necessary to form gradients between solvents of varying viscosities.

*Figure 5 Helium Panel*


**Wash Bottle Pressurization System**

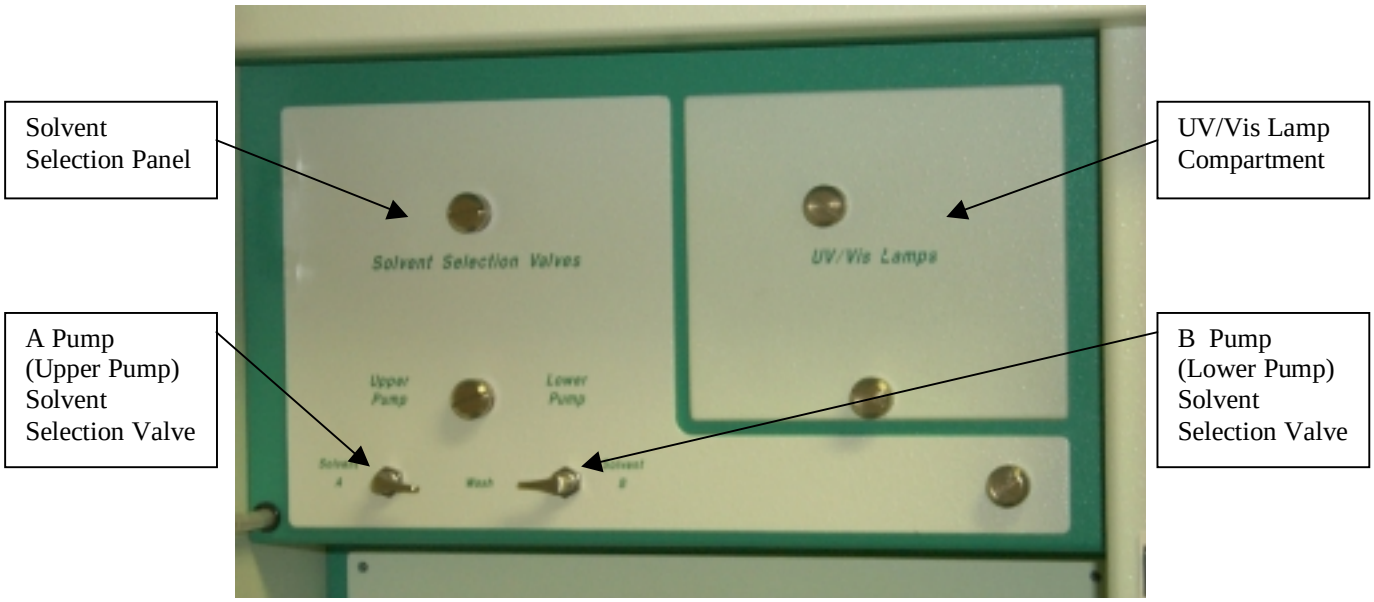
The delivery of Helium to the wash bottle is constant at 6 PSI. There is no shutoff valve for this gas line. ***When replacing the wash solvent, depressurize the wash bottle prior to opening the bottle***. To depressurize the wash bottle, place the Helium valve on the Helium panel in the "Off" position (*Refer to Figure 5)*, turn off the Helium source to the instrument, and then vent the bottle by loosening the Delrin plug on the cap insert.

The solvent in the wash bottle serves several functions. The wash solvent washes behind the pump piston seals at a flow rate of 5-10 µl/min to clear away any particulate matter or salts that may damage the seals or pistons. The wash behind the pump pistons continually flows to waste, and any leaks that occur because of worn primary piston seals are also vented to waste. Note: The piston wash continually flows at 5-10 µl/min as long as the wash bottle is pressurized with a Helium source. ***The Wash Solvent level should be checked regularly to ensure that the wash bottle solvent is never depleted, as this can cause rapid loss of Helium from the source and may damage the pistons and piston seals.*** Another function of the wash solvent is to flush the system of potential harmful mobile phases or to clean the system prior to shutdown. To flush the system with wash solvent, point the solvent selection valves (*Refer to Figure 6, page 17)* inward toward the wash position.

## High Pressure Pumps

The system contains two pumps (*Refer to Figure 2, page 12*), an upper pump (Pump A) and a lower pump (Pump B). Solvents are delivered to the pump heads from either the solvent bottles on the door or the wash solvent on the right side instrument panel. The solvent from the "A" bottle travels through the left side solvent select valve (*Refer to Figure 6*) into the upper pump head. Solvent from the "B" bottle travels through the right side solvent select valve into the lower pump head. If the solvent select valves are positioned inward toward "Wash", then the wash solvent travels through the pump heads. If the solvent select valves are in the upward or "Off" position, no solvent is going to the pump head. The solvent select valves interrupt solvent flow to the pump heads that occurs due to Helium head pressure on the solvents. Positioning the valves in the "Off" (upward position) allows the operator to change the column, injector loops, mixer or pump heads without depressurizing the A/B Solvent bottles. ***Never turn the solvent select valves to the upward position if the pumps are running***. Operating the pumps without solvent supplied to the pump heads may cause permanent damage to the pump head seals and pistons.

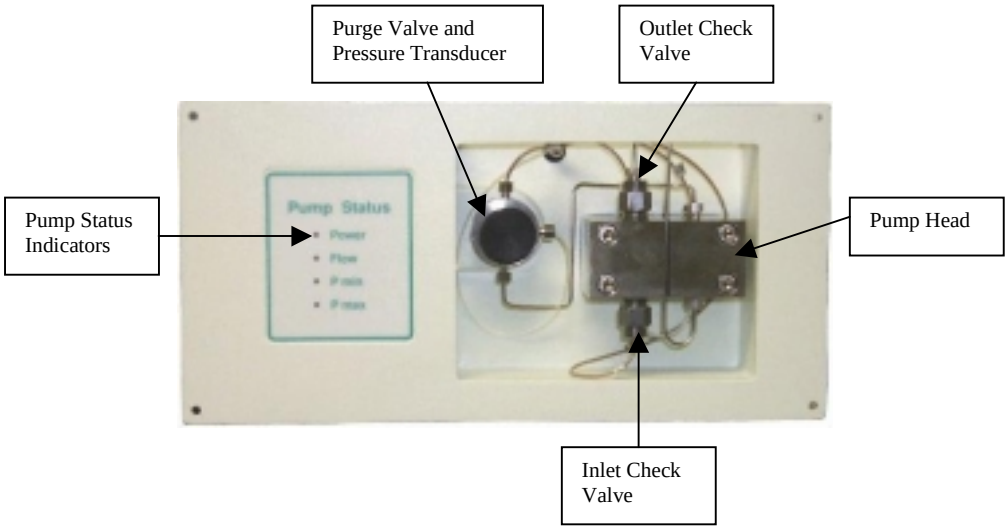
*Figure 6 Solvent Select Panel & UV/Vis Panel*




The pump status indicators are on the left portion of each pump faceplate (*Refer to Figure 7, page 18*). The power indicator LED (light emitting diode) should be on whenever the power to the instrument is on. The pump status flow indicator LED should be lit when the pumps are operating. The Pmin (pressure minimum) indicator light will flash when a pump error has occurred due to low system pressure. The default Pmin value is Zero, therefore, the pumps will continue to run even if the purge valves are open or the system has developed a leak. The Pmax (pressure maximum) indicator light will flash when a pump error has occurred due to high pressure. The default Pmax is 5000 PSI, which should stop

the pumps without causing system leaks. Please contact Michrom BioResources, Inc. before attempting to change the default Pmin and Pmax settings.

*Figure 7 Pump Skid Faceplate*




The pump heads are located to the right of the purge valve (*Refer to Figure 7*). The pump heads are mounted onto the pump drive by four Allen head screws. The MAGIC 2002™ offers either analytical heads for gradient flows between 0.1 and 5 ml/min or microbore heads for gradient flows between 20 and 1000 µl/min. Capillary flows are also achieved using the microbore pump heads in conjunction with a pre-column flow splitter for gradient flows between 0.1 and 10 µl/min. The two solvent lines located on the back of each pump head (top and bottom) are the wash solvent lines for solvent flow behind the pump head piston seals. The inlet solvent line from the solvent select valve is located on the lower left side of the pump head. An inlet check valve is the interface between the inlet line and the pump head. The outlet check valve is located on the upper left side of the pump head and is the solvent path to the lower right side of the pump head. Solvent exits the pump head from the upper right side and is directed into the bottom of the purge valve via a stainless steel tube.

The purge valve (*Refer to Figure 7*) houses the pressure transducer. There are three solvent lines attached to the purge valve. The stainless steel inlet line from the pump head is located on the bottom of the purge valve. The PEEK line to the mixer is located on the top of the purge valve. The Teflon line on the right side of the purge valve is routed to the waste container. The purge valve in the closed position (completely clockwise until finger tight) allows solvent to flow through the PEEK line exiting the top of the purge valve to the mixer. The volume from the purge valve to the mixer is approximately 65µl. The purge valve turned counter clockwise to the open position allows for the venting of solvent and air to waste via the Teflon line exiting the purge valve to the left. Further discussion on displacing air bubbles from the pumps using the purge valves is found in Chapter

2 (Section I) under System Startup. It is not necessary to fully open the valve (multiple full revolutions of the valve) during purging.

## Variable Volume Mixer

Solvent passes from the pumps to the dual stage, variable volume mixer (*Refer to Figure 8, page 20*) located below the column oven compartment adjacent to the 10-port injector valve. The first stage is a static vortex mixing tee that is followed by a high pressure static mixer cartridge. The cartridges are designed to provide effective gradient delay volumes with effective mixing to cover the gradient range of the MAGIC 2002™ *(Refer to Table 1)*. The housing for the mixer cartridge is mounted onto the valve/actuator panel of the instrument for easy accessibility.

*Table 1 MAGIC™ Mixer Cartridges*

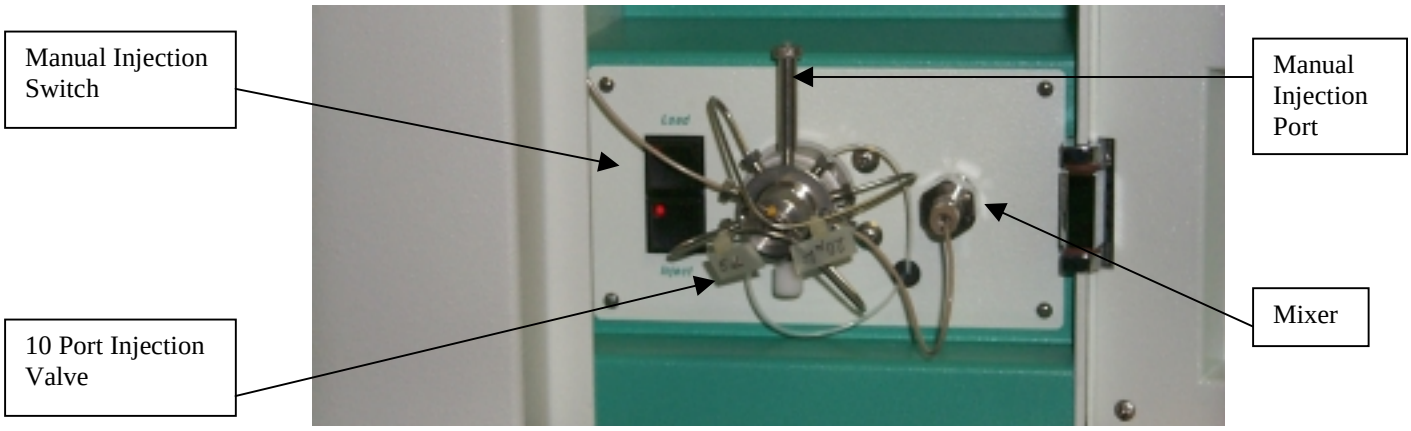
| Cartridge Size | Physical Volume | Total Sweep Out Volume | Effective Flow Rate Range |
|---|---|---|---|
| 50 µl | 18 µl | ~50µl | 20-100µl/min |
| 100 µl | 36 µl | ~100µl | 40-200µl/min |
| 200 ul | 72 µl | ~200µl | 100-500µl/min |
| 500 µl | 180 µl | ~500µl | 500-2500µl/min |
| 1000 µl | 360 µl | ~1000µl | 500-5000µl/min |

To reduce baseline noise and maximize reproducibility for slow (<1%/min) high sensitivity gradient runs, use a larger mixer volume. For fast (>1%/min) gradients and faster system re-equilibration, use a smaller mixer volume.

## Injector Valve

The flow input from the mixer routes to the 10-port injector valve (*Refer to Figure 8, page 20*). This valve is for sample loading and provides additional ports for flexibility in sample handling. The standard loop volume for microbore flows is 20 µl, although loops ranging from 0.5-1000µl are available. Michrom BioResources, Inc. trap cartridges for concentrating and desalting samples (see Trapping Options in Chapter 3) can also be used as sample loops. There is an electronic switch on the left side of the valve for manual load/inject positioning of the valve. The injector sensing/positioning is also controlled from the software via both Control/Instrument Status and Method/Instrument Setup menus. The injector position and port connections can also be observed on the Control/Instrument Status screen. Samples can be manually loaded with a syringe through the manual injection port located on valve port number one. Valve port one is located on the top of the valve and is accessible with the door closed. The volume from the mixer to the end of the column inlet line with a 20 µl loop is approximately 40 µl.

*Figure 8 Injection Valve Panel*




## Column Compartment and Flow Cell

The column compartment is a temperature controlled forced-air heated oven. The column oven compartment door is located in the top portion on the front right hand side of the MAGIC 2002™. The compartment door is hinged on the bottom (*Refer to Figure 9, page 21*) so that the top of the door moves out and down to allow access to the column, UV detector photodiode and flow cell assembly. The opening of this door causes the temperature controller to shut off. The compartment is designed to handle various internal diameter columns and column lengths up to 250 mm. Conventional columns are connected to the flow cell assembly using a Michrom BioResources, Inc. Standard Column Adapter. Michrom BioResources, Inc. columns are connected directly into the bottom of the flow cell assembly.

The flow cell assembly (*Refer to Figure 10, page 21*) consists of an outer diode (secured to the mounting plate with two thumb screws), mounting plate, flow cell holder, flow cell and flow cell outlet line. The flow cell is held in the holder by the outlet line and a locking nut. An analytical system consists of a 5 mm path length flow cell (2.4 µl volume) with a 0.007 inch ID outlet line (5 µl volume). The microbore assembly consists of a 2 mm path length flow cell (0.3 µl volume) with a 0.003 inch ID output line (1 µl volume). A capillary assembly consists of a 2 mm path length flow cell (0.02 µl volume) with a 0.002 inch ID outlet line (0.6µl volume). The flow cell output line is attached to the waste line via the stainless steel bulkhead union located on the ceiling outside the column compartment. Alternatively, the flow cell outlet line can be routed to an auxiliary detector and/or fraction collector by attachment through the access port in the upper right side using a zero-dead-volume union or tee (*See Spare Parts List, page 10).*

*Figure 9 Column Compartment (Opened)*

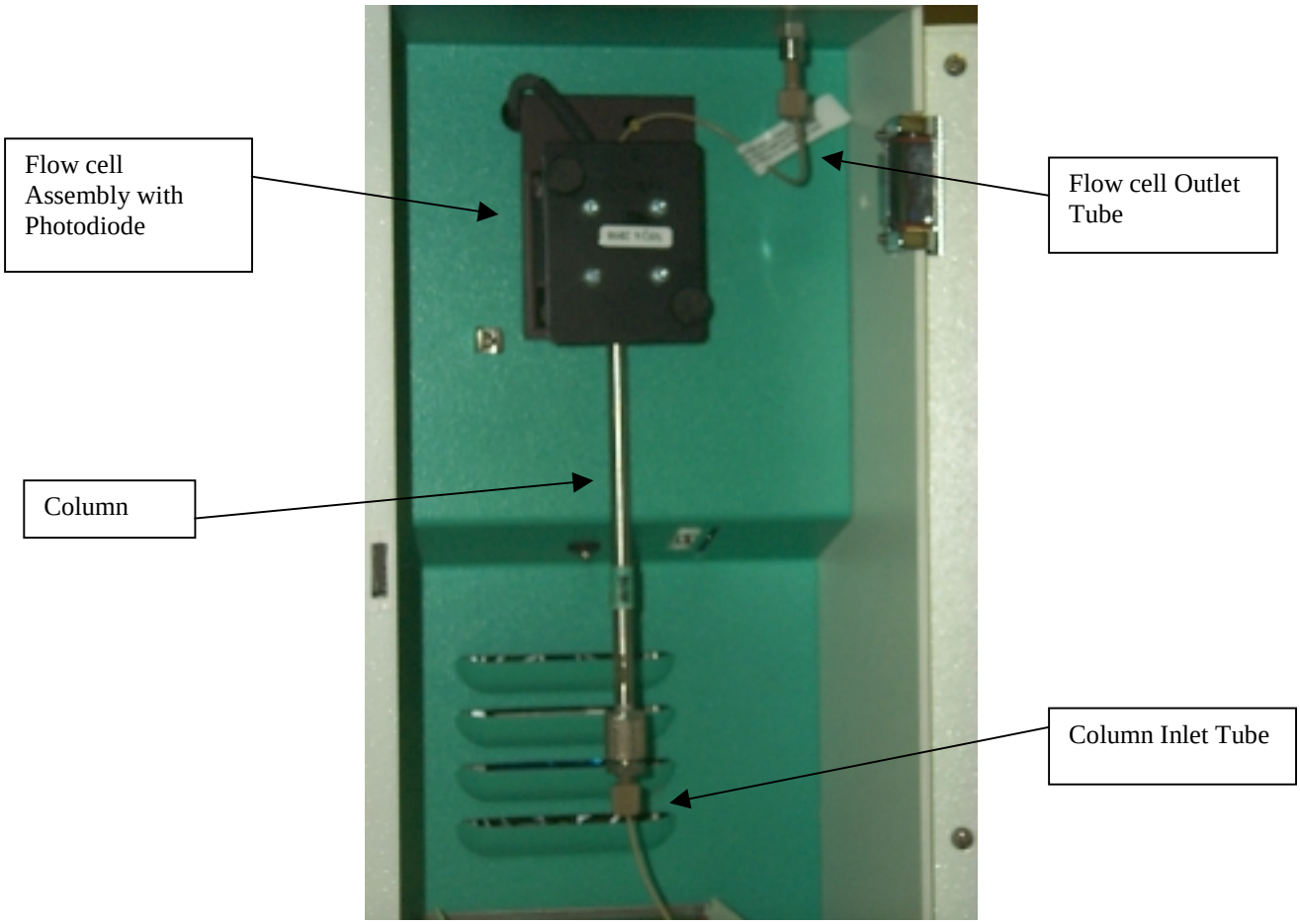
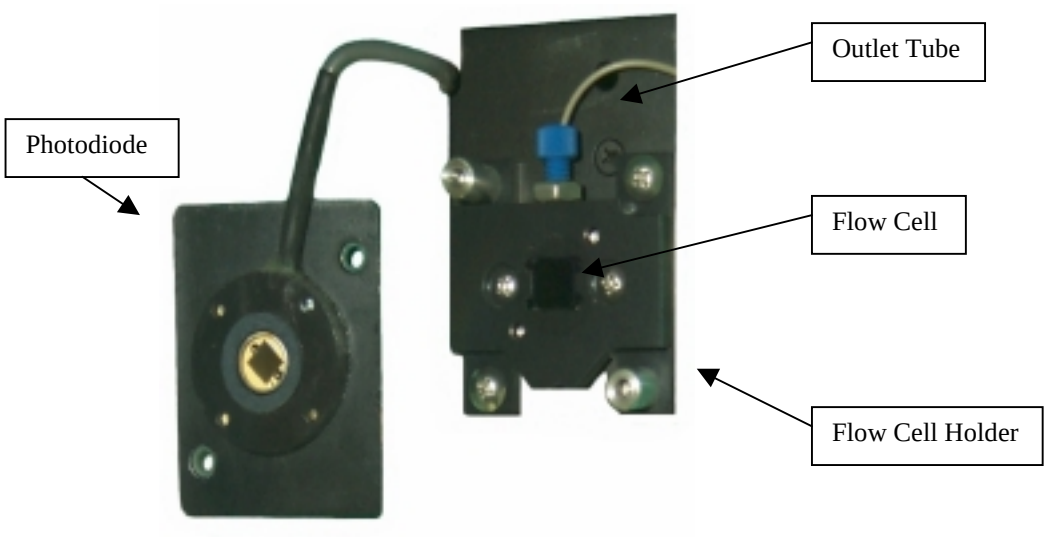


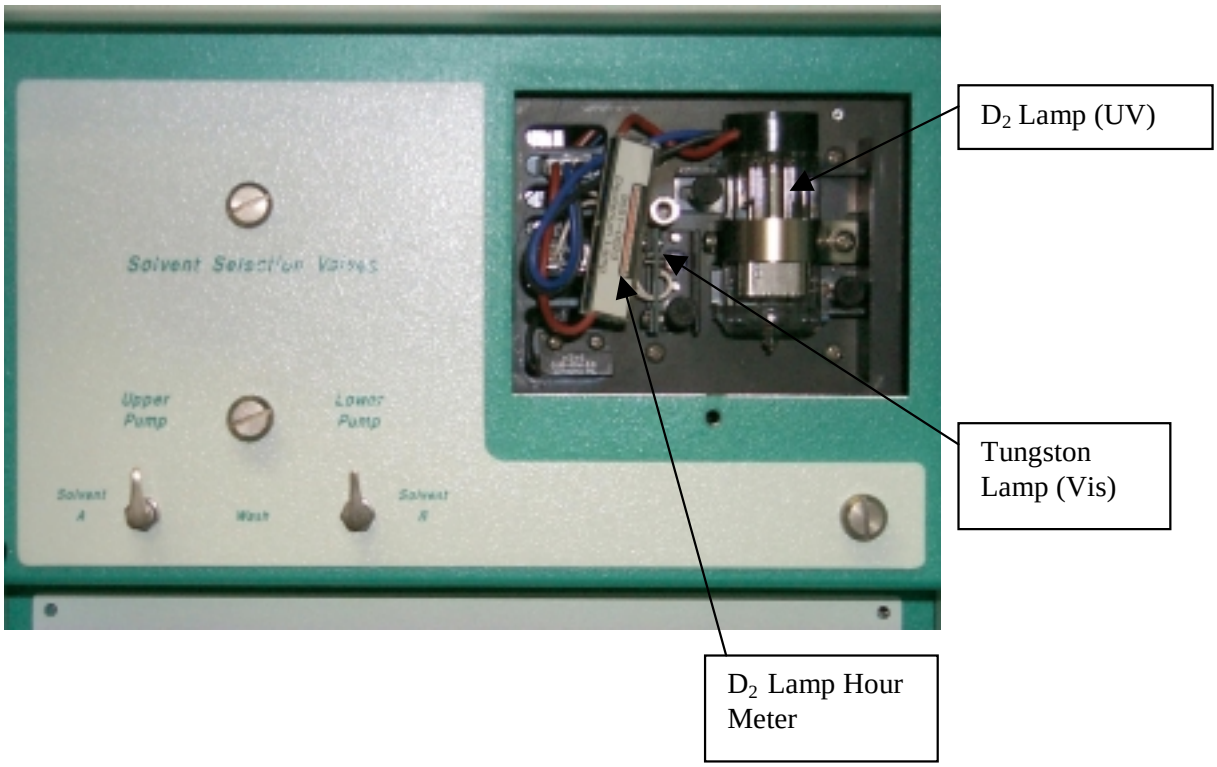

*Figure 10 Flow Cell Assembly with Photodiode*



## UV/Vis Detector

The UV/Vis detector is located above the upper pump (A solvent pump) in the pump compartment. Removing the UV/VIS compartment front cover allows access to the UV lamp compartment (*Refer to Figure 11*). The UV/Vis lamp compartment houses the pre-aligned Deuterium (D2) lamp (UV) and Tungsten lamp (Vis). The MAGIC 2002™ operates in either single or dual wavelength modes. The lamp compartment cover must be installed for the lamp to operate. If the cover is removed, the lamp will turn off. The lamp will restart (re-fire) and the wavelength will reset when the door is reinstalled.

*Figure 11 UV/Vis Lamps Compartment (Opened)*



# CHAPTER 2

# BASIC OPERATION

Initial installation of the MAGIC 2002™ is normally performed by Michrom BioResources, Inc. personnel or their authorized agents. Instrument installation includes power, cabling, Helium, pump heads, flow cell, column, solvent bottles, and accessories. The gas and solvent connections, system controller (data system), and accessory connections are made on site and system integrity is verified. If the MAGIC 2002™ system is removed or shipped to a new location, note the cabling configuration for proper reinstallation. If problems arise after moving the MAGIC 2002™, contact Michrom BioResources, Inc. or the authorized representative in your area for technical support. Note: The MAGIC 2002™ is shipped with two lock-down screws for each pump fastened to the back of the instrument. These screws are normally removed during installation to allow access to the pump compartments from the front of the instrument. ***Note: Although the system can be moved without these lock-down screws in place, it should not be shipped without replacing these lock-down screws***.

## I. System Startup

### Column Installation

Michrom BioResources, Inc.'s microbore columns (0.5, 1.0 and 2.0 mm ID) are designed to thread directly into the Flow Cell Holder (*refer to Figure 9, page 21*). Michrom BioResources, Inc.'s capillary columns (0.1, 0.2 and 0.3 mm ID) are designed to thread into a specially designed Flow Cell Inlet tube. A standard column adapter (P/N 602-25026-01) should be used with all non-Michrom BioResources, Inc. columns that use conventional 10/32 end fittings. The column inlet line should be connected to the column inlet (or guard column inlet) or be directed to a waste reservoir prior to pressurizing the MAGIC 2002™ solvents with Helium. Helium head pressure (6 PSI) will cause solvent flow with no system back pressure if the solvent select valves are in the A/B or wash postions. ***Note: It is recommended that all pump flows be turned off whenever installing or changing a column.***

### Solvent Sparging and Detection of Helium Leaks

Confirm that all solvent containers have the appropriate solvents and are properly sealed. The solvent bottle cap inserts form a seal between the Viton o-ring and the bottle tops when pressurized with Helium. Inspect the tops of the bottles for chips and/or cracks and ensure that each o-ring is properly seated in the groove on

the underside of the cap insert prior to tightening the caps on the bottles. The system is shipped with plastic coated bottles with plastic pouring rings removed from the top. If other bottles are to be used with the MAGIC 2002™, verify they are clean and crack/chip free before use. Although the Viton o-rings are very inert, some organic solvents can cause the o-rings to swell in size. If the o-ring does not fit into the grove in the cap insert, it should be removed and replaced with a new o-ring. If the swollen (oversized) o-ring is not torn or cut, it can be stored in a drawer and will return to its original size in a few days. The MAGIC 2002™ is shipped with 3 spare o-rings.

Turn on the Helium source to the instrument. Helium should be regulated to the instrument between 20 and 200 PSI. This pressure is then regulated within the instrument to 6 PSI. Please note that once the Helium source is on, Helium will be delivered to the wash Bottle. To allow Helium to be delivered to the Solvent A and Solvent B bottles, open the Helium valve located above the solvent bottles (*Refer to Figure 5, page 16*). To open the valve, place the valve in the On position. A moderate bubbling of gas should be observed in the A and B bottles with the opening of the Helium valve. Prior to pressurizing the solvent bottles, sparging should be performed. To sparge the bottles (degas solvents), simply select Sparge/Vent on the Sparge/Vent valve next to the Helium On/Off valve. After one to three minutes of sparging, the Sparge/Vent valve should then be turned to Pressurize. While the solvent bottles are pressurizing, the instrument should be checked for Helium leaks. Check for gas leaks around the solvent bottle caps and all associated fittings. Also, check for Helium leaks around the inlet fitting to the instrument, the Helium On/Off valve and the Sparge/Vent valve. The system is completely pressurized when no more bubbling is observed in the bottles. This may take as little as 10 minutes or as much as several hours depending on the type of solvents used, and the solvent level in each bottle.

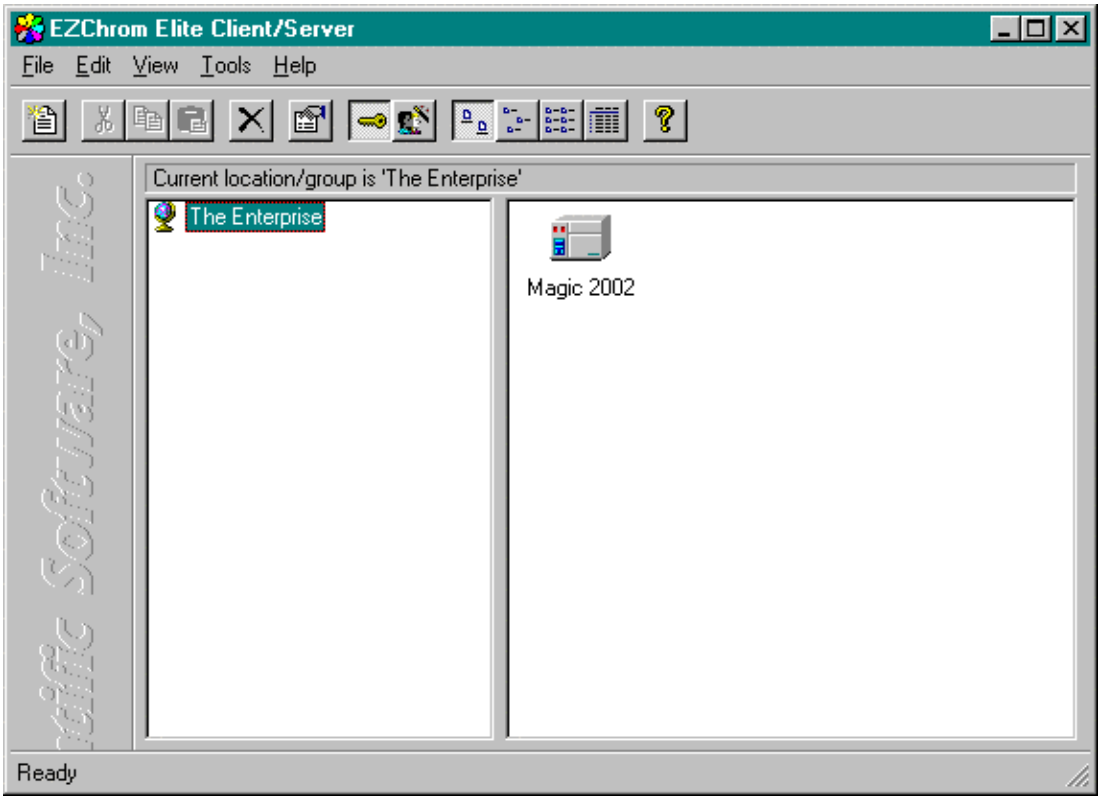
**Solvent Flow to the Pump heads**

Prior to turning the pumps on, confirm that there is solvent flow to the pump heads. To check for flow at the pump heads, adhere to the following procedure. Turn the Solvent Select valves (*Refer to Figure 6, page 17*) to the desired solvent, open the purge valves (*Refer to Figure 7, page 18*) and observe the Teflon waste line exiting the purge valve to the right for flow of solvent and/or air bubbles. Allow the Helium head pressure to force the solvents through the pumps for 3-5 minutes before closing the purge valves. This procedure should be followed for the A/B solvents and/or the wash solvent any time the solvent inlet lines have gone dry. The above process is not necessary during routine solvent changes or when refilling the solvent bottles.

## Powering up the PC & the MAGIC 2002™

After solvent flow has been established to the pump heads, power can be supplied to the computer and instrument. The main power switch to the instrument can be found on the right side panel toward the rear of the instrument (*Refer to Figure 4, page 14*). Allow 2-3 minutes for the detector to initiate diagnostic routines before starting the MAGIC 2002™ control software on the computer. To open the control software, double click on the MAGIC 2002™ icon. At the EZChrom Elite Client/Server screen (*Refer to Figure 12)* double click on MAGIC 2002™ to access the instrument control and data acquisition windows.

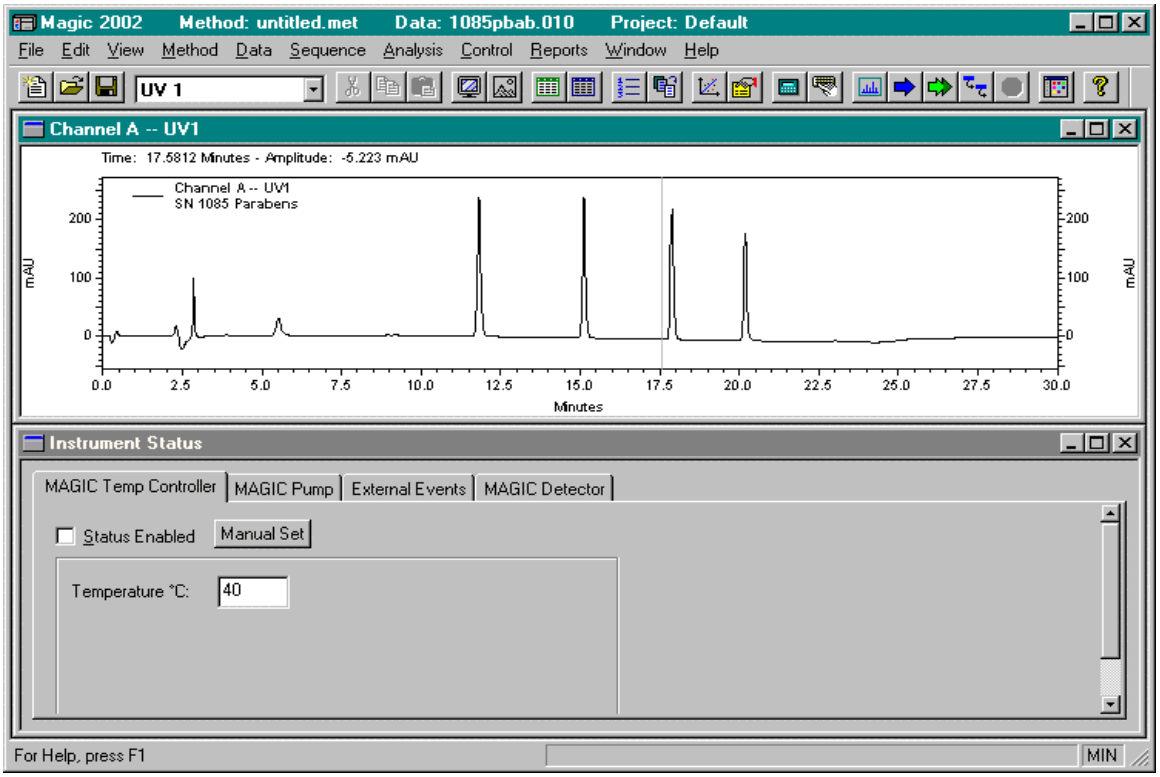
*Figure 12 EZChrom Elite Client/Server Screen*




## Initial System Operation

The initial control of the MAGIC 2002™ is through the Instrument Status window. To access the Instrument Status window, click on Control and select Instrument Status (*Refer to Figure 13, page 26*). The solvent select valves are normally in the Off position prior to initial system startup and need to be manually changed to select wash solvent or solvents A and B prior to enabling the pumps using the Control/Instrument Status Magic Pump window (*Refer to Figure 24, page 34*). ***Note: It is necessary to click on status enable to verify pump head type prior to running or purging the pumps.***

*Figure 13 Instrument Status/Preview Screen*




## Purging Pumps to Remove Air

The pump purge flow rate for each pump is 1 ml/min for microbore pump heads and 5 ml/min for analytical pump heads. The pumps are purged to remove air in the solvent lines and to replace wash solvent with gradient solvents to the column inlet. The minimum purge time required to displace system solvents and/or air bubbles is approximately 30 seconds. With solvent flow verified to the pump heads (*Refer to page 24 "Solvent Flow to the Pump heads")*, close the purge valves, ensure solvent select valves are set to the wash solvent position and direct the column inlet line to a waste container. The system purge control is found on the Magic Pump tab in the Control/Instrument Status window (*Refer to Figure 24, page 34*). Disable the Status Enabled (if checked) and click on the Pumps On, Purge On for both pumps A and B then, click on Manual Set. A warning box will be displayed for each pump to open purge valves as a precautionary reminder in case the column is in the solvent pathway. Opening the purge valves is not required if the column inlet line is removed from the column prior to purging. With both pumps purging at 1.0 ml/min, pressure should build to between 300-600 PSI and a continuous stream of solvent should be exiting the column inlet line (Note: Pressure can be viewed from the Magic Pump Status screen, or if running with the 4-channel data system option, a graphical display of pressure can be obtained on Channel D by using the Preview button on the tool bar).

If the flow and/or pressure are inconsistent, the A and B purge valves should be opened and closed until no air bubbles are observed in the purge valve exit line and the flow and pressure stabilize (Change in pressure should be no greater than ±50 PSI). Once good flow is established for both pumps with wash solvent, the purge/prime procedure should be repeated for solvents A/B. After purging is complete, close the purge valves, and return the pumps to the control of the method by removing the check marks from the Purge On for both pumps, setting the flow rate and %B, clicking on Manual Set and then clicking on Status Enabled. At this time, the tubing can be reconnected to the column inlet and the column allowed to equilibrate prior to running samples.

**System Pressure Stabilization**

Allow the system to equilibrate at the optimum column flow rate at 50% B until pressure and baseline are stable (5 to 15 minutes). Set the %B to 95% for 5 to 10 minutes to flush the column, then set the %B to the initial conditions for the method to be executed (i.e., 5%B) and wait until the pressure and UV baselines are stable.

# II. Operating the MAGIC 2002™

The MAGIC 2002™ control and data acquisition system is based on the EZChrom *Elite* Client/Server platform. The purpose of this section is to provide step-by-step instructions for creating or editing a MAGIC 2002™ method. This section also describes how to use the Instrument Status window for direct access and control of each of the MAGIC 2002™'s components. If the MAGIC 2002™ was purchased with only the control software, the information in this section will provide the necessary information for running the instrument. If the system was purchased with the control and data system option, detailed explanations of all the data system functions can be found in the EZChrom *Elite* on-line manual on the CD-ROM.

**Creating or Editing a Method**

The method is edited in the Instrument Setup window. To access the Instrument Setup window, click on Method and select Instrument Setup or click on the  button on the toolbar. Each component of the MAGIC system has a separate tab for entering data (*Refer to Figures 14-19, pages 28-30*). To create or edit a method, click on the appropriate tab, position the cursor in the appropriate field and enter the appropriate data. An example of a method typically used for a 1.0mm ID column is provided on the following pages. The values that can be edited are in white and items that cannot be edited are gray. After creating a new method or editing an existing method, save the method prior to running it in the single run or sequence run mode. Methods will be saved automatically with a *.met* extension unless otherwise specified.

*Figure 14 Instrument Setup of Temperature Controller Screen*

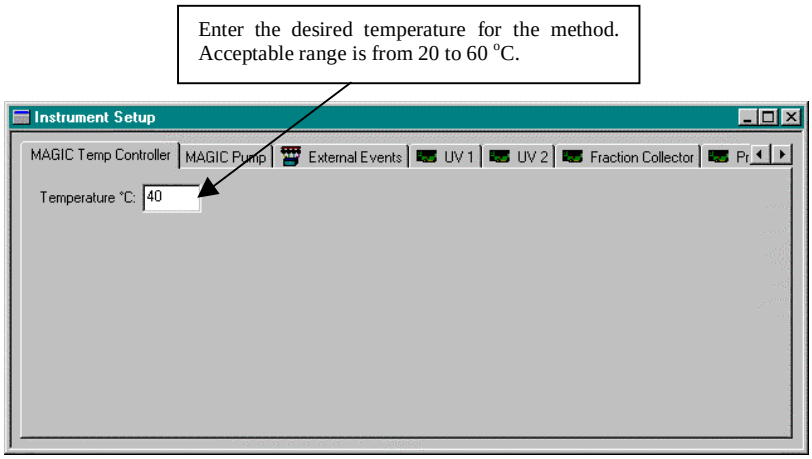



*Figure 15 Instrument Setup of Pumps Screen*

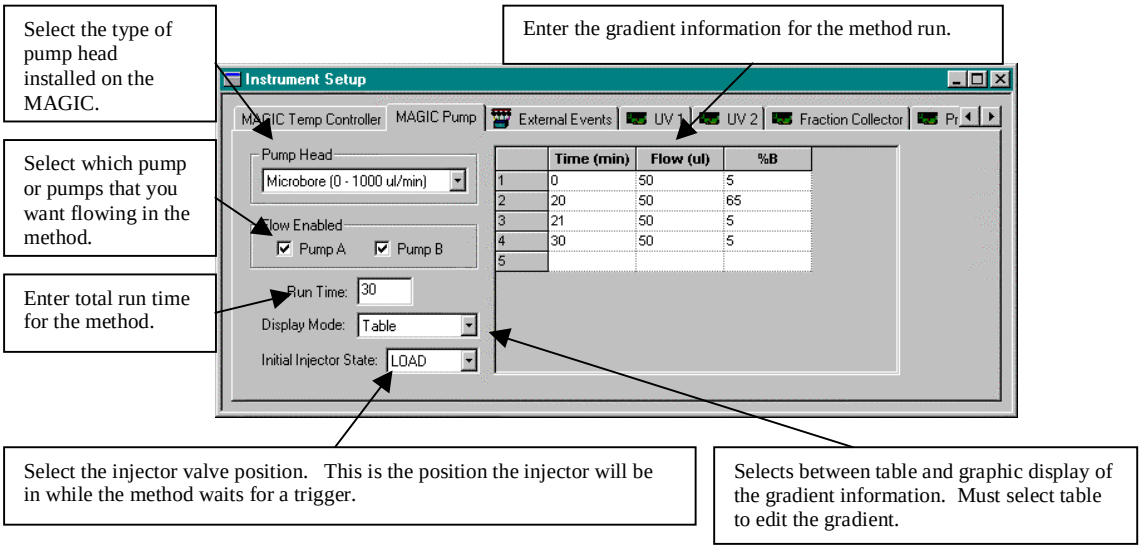


*Figure 16 Instrument Setup of External Events Screen*

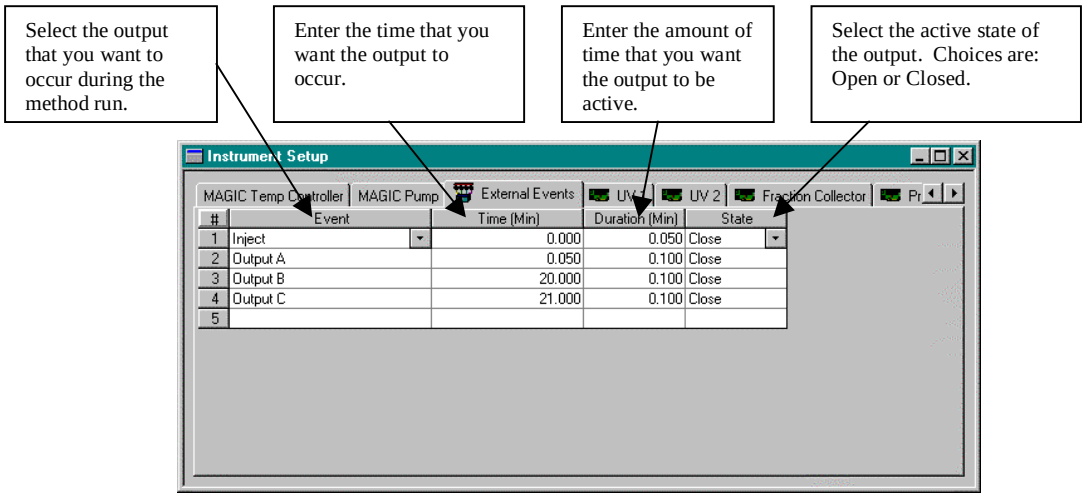
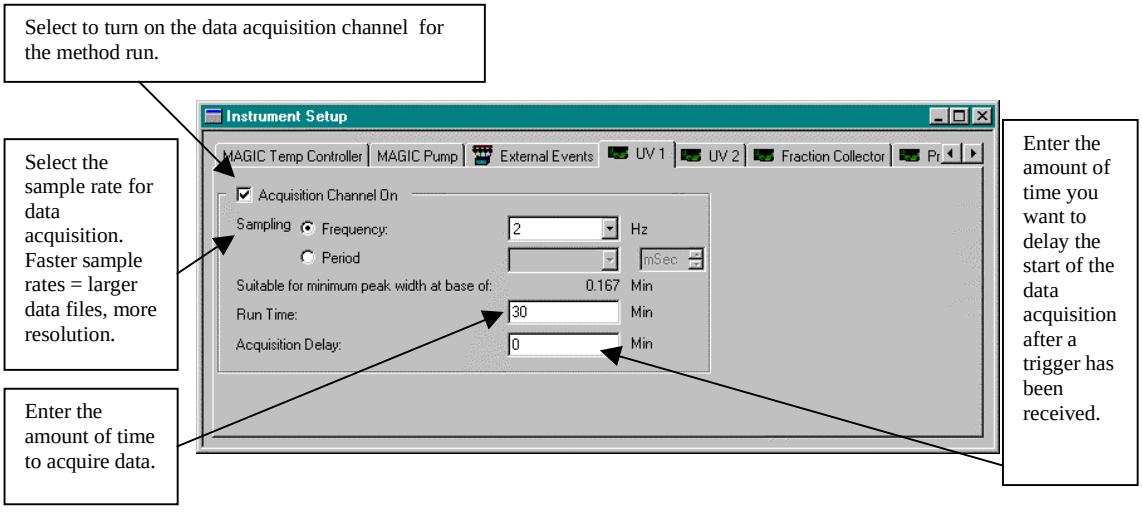



*Figure 17 Instrument Setup of Data Acquisition Channels Screen (Same for all channels)*




*Note: The screens for data acquisition will only be displayed in the method if the data system option was purchased as part of the MAGIC™ control software*

*Figure 18 Instrument Setup of Detector Screen*

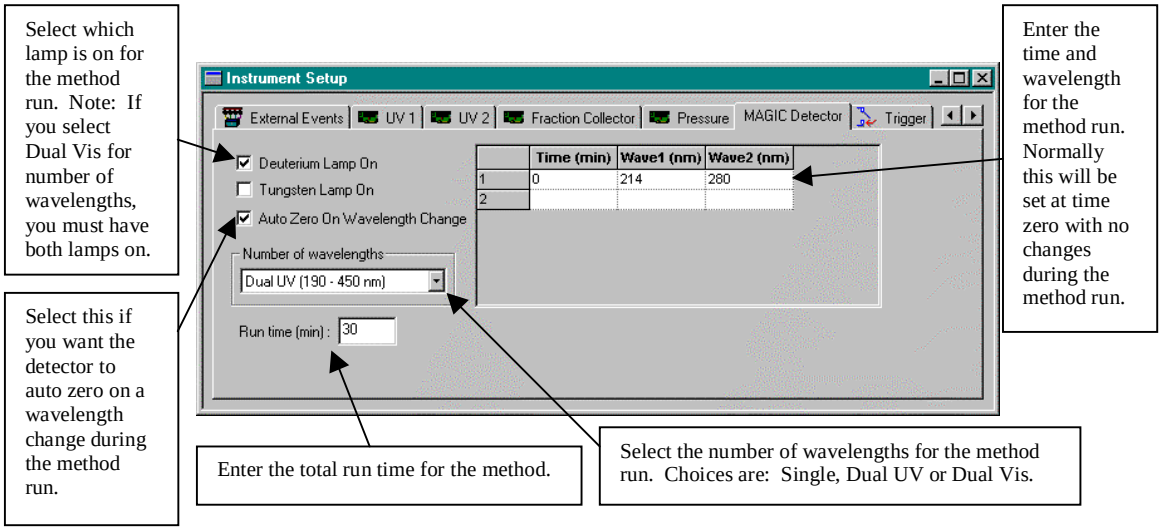
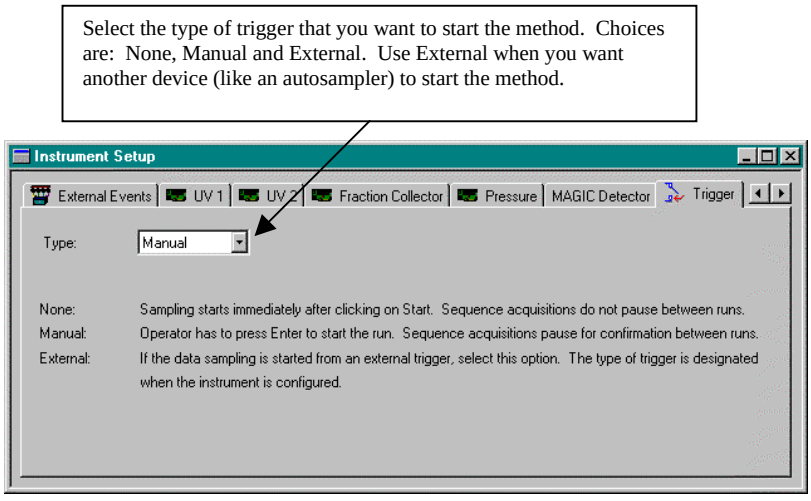


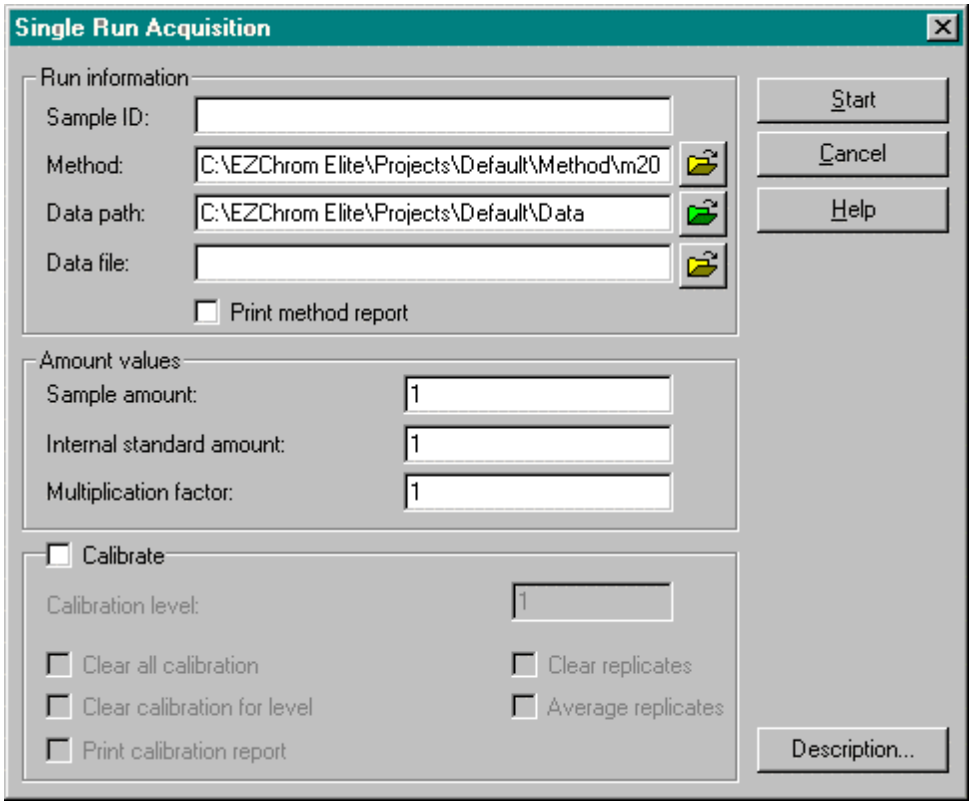

*Figure 19 Instrument Setup of Trigger Screen*



## Single Run

A single run can be performed by clicking on the dark blue single arrow on the tool bar (*Refer to Figure 13, page 26).* This will bring up the Single Run Acquisition Screen (*Refer to Figure 20).*

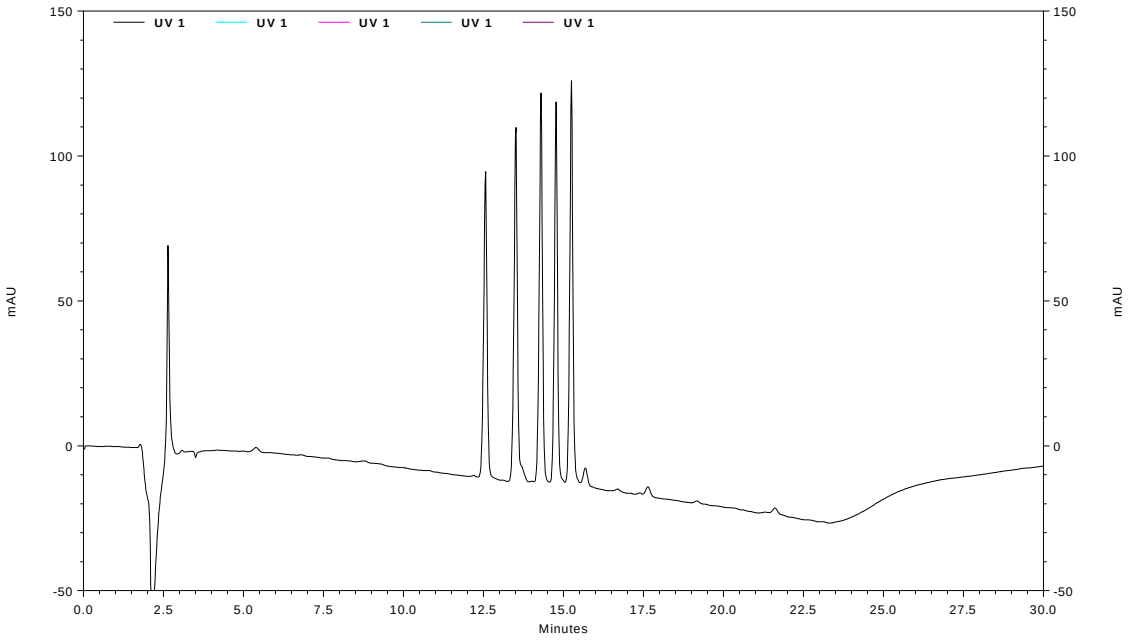
*Figure 20 Single Run Acquisition Screen*




To verify that the system is operating properly, analyze a standard using the appropriate flows for the column of choice (*Refer to Chapter 3, Section I, page 41).* If a 1.0mm ID column is in-line, simply use the method as created in figures 14-19. Prior to starting a run, flush the sample loop with A Solvent to ensure that the loop is free of organic solvent greater than 5%. Load a syringe with the appropriate amount of test mixture, and then load the syringe contents into the sample loop (*Refer to Chapter 3 Section I for column capacity).* For example: If a 1.0mm ID peptide column is in-line, load the syringe with 5µl of A Solvent and 10µl of the 10ng/µl Synthetic Peptide Test Mix (Part Number 910-00002-02), and then load the loop with the syringe contents. Once the sample loop has the appropriate contents, start the run. An example of a typical test chromatogram on

a 1.0mm ID peptide column (Part Number 900-61211-00) is provided below (*Refer to Figure 21).*

*Figure 21 Michrom BioResources, Inc. Peptide Standard Chromatogram*

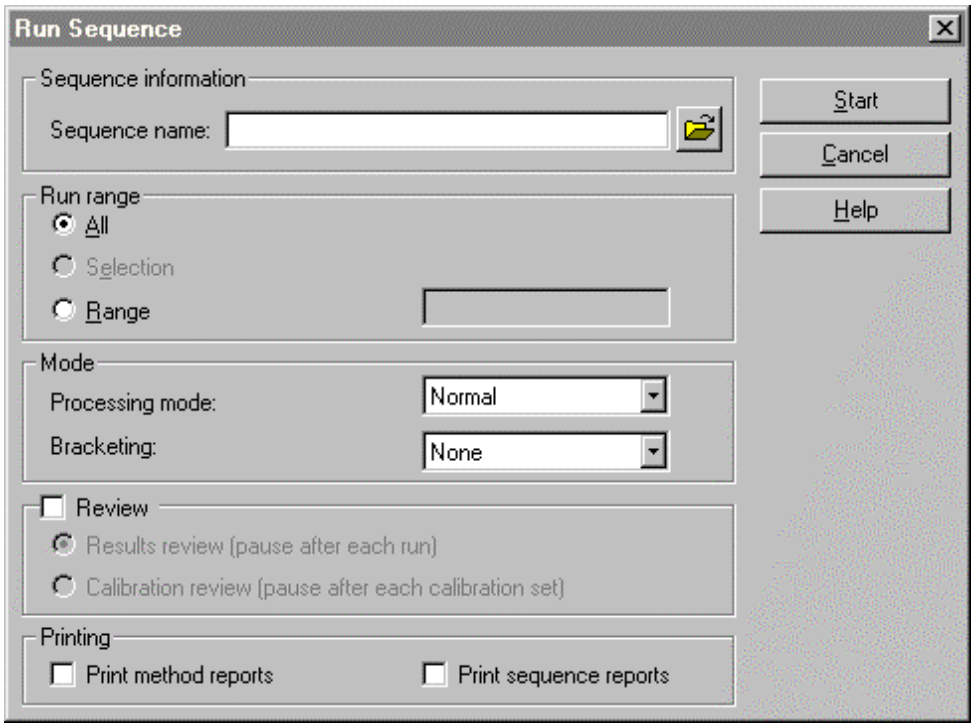



**Column Type** ..... **MAGIC MS C18**
**Size** ..... **5µ 200A**
**Part #** ..... **900-61211-00**
**Dimensions** ..... **1.0 X 150mm**
**Gradient** ..... **5%B-65%B in 20 min**
**Solvent A** ..... **2/98/0.1% ACN/$H_2O$/TFA**
**Solvent B** ..... **90/10/0.09% ACN/$H_2O$/TFA**
**Flow Rate** ..... **50µl/min**
**Test Mix** ..... **Part # 910-00002-02, Synthetic Peptide Test Mix**

## Sequences

Sequences link individual methods together for multiple, automated runs. Sequences can be created method by method or by multiple runs of the same method. To create a sequence, click on File (*Refer to Figure 13, page 26)*, choose Sequence, then choose New Sequence and follow the Sequence Wizard. Save the sequence once the wizard is finished. To run a sequence, click on the green double arrows button on the tool bar (*Refer to Figure 13, page 26).* This will pull up the Run Sequence Screen (*Refer to Figure 22, page 33).* Please refer to the EZChrom on-line manual on the CD-ROM for details.

*Figure 22 Run Sequence Screen*




## Instrument Status

The Instrument Status window is where direct control of each of the MAGIC 2002™ components can be performed. Manually setting individual parameters can also be performed, as well as monitoring the status of the current operating conditions during a single method or sequence run. To open the Instrument Status window, click on Control (*Refer to Figure 13, page 26)* and select Instrument Status. Each component of the MAGIC 2002™ system has a separate tab (*Refer to Figures 23-26, page 34-35)* in which to enter data and/or monitor status. If no check mark is present in the Status Enabled box, data can be entered into any white field. When the Manual Set button is clicked, all of the values in the fields are sent to the instrument and take effect immediately. When there is a check mark in the Status Enabled box, the fields reflect the current instrument conditions. The Status Enabled box should always be checked during a single method or sequence run. Although the Manual Set function can be used to change conditions manually during a run, these changes will not be saved with the method or data file, making it difficult to interpret results at a later time. If a method run is not going as expected (no peaks or sample elutes at time zero), the method should be stopped and Manual control used to change, flush out and re-equilibrate the system prior to restarting a run.

*Figure 23 Instrument Status of Temperature Controller Screen*

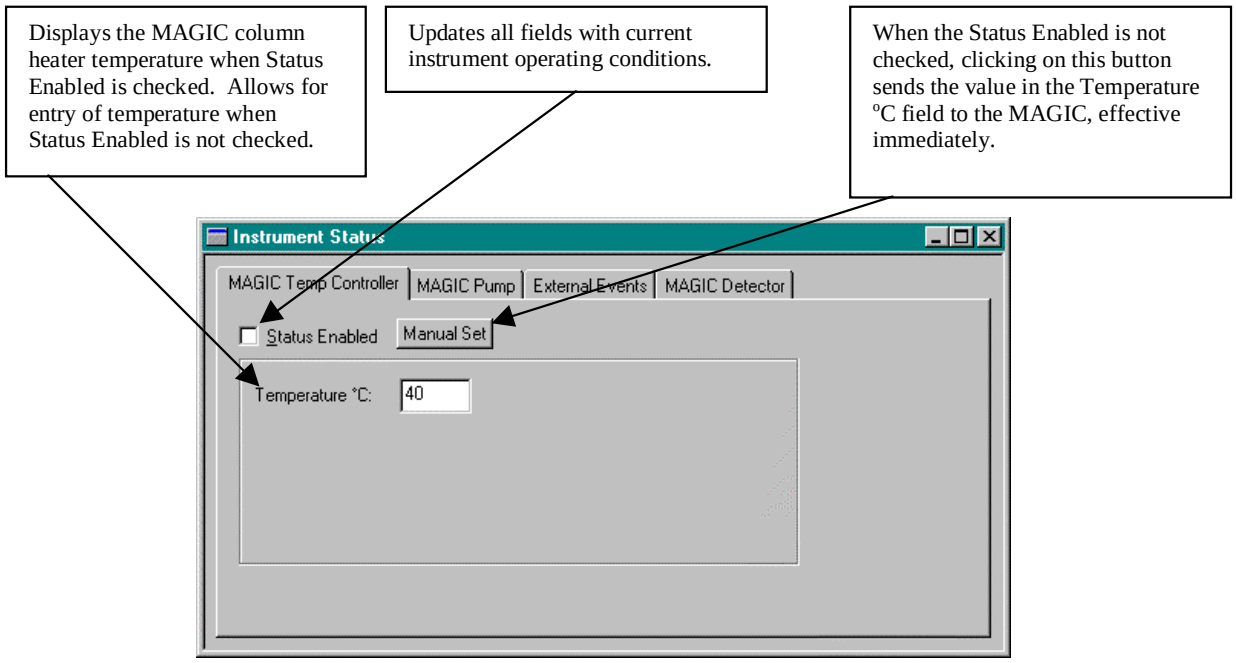



*Figure 24 Instrument Status of Pumps Screen*

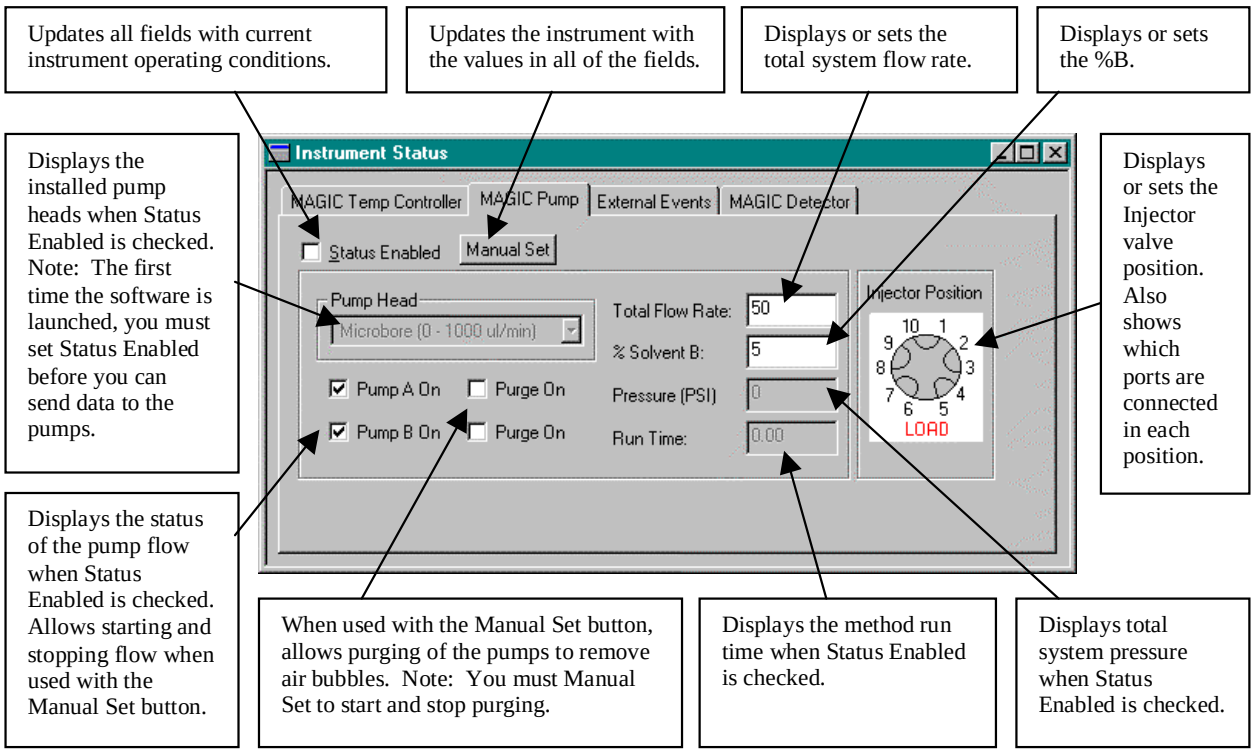


*Figure 25 Instrument Status of External Events Screen*

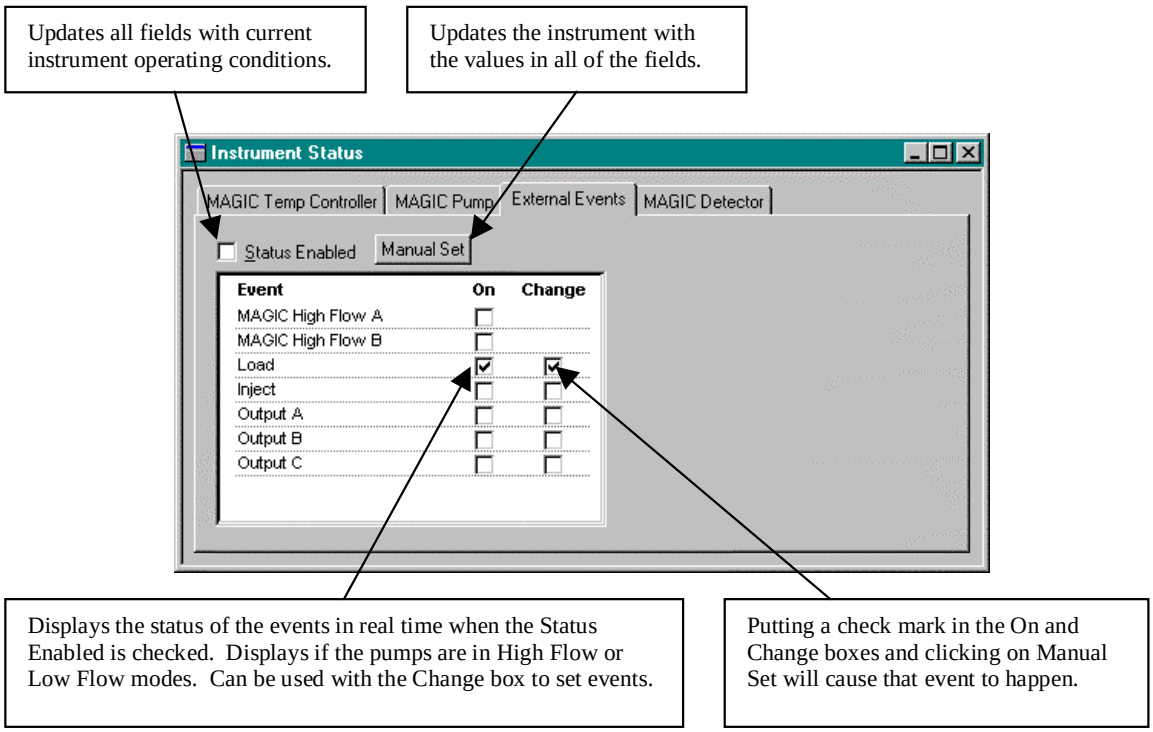
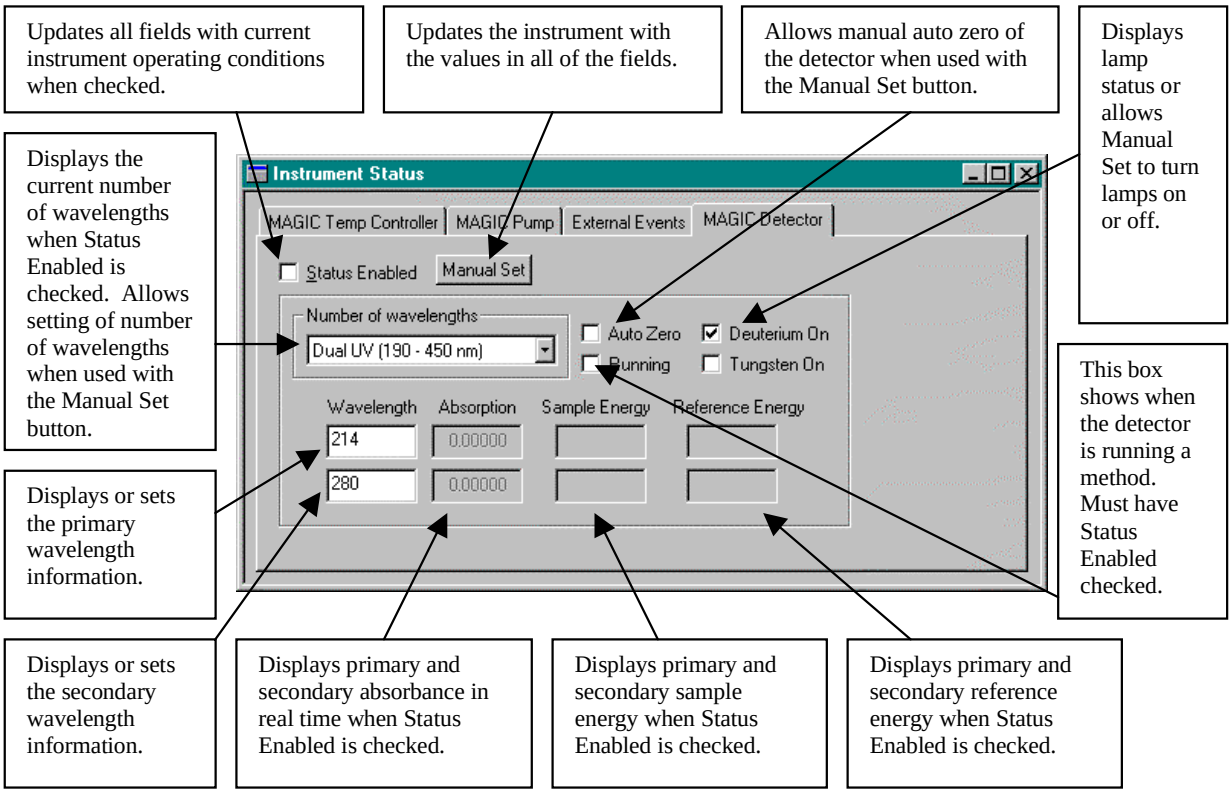



*Figure 26 Instrument Status of Detector Screen*



# III. System Shutdown

When the system is not in use, an orderly shutdown procedure should be followed to help maintain the instrument.

### Daily Shutdown

At the end of the day, run the shutdown procedure. If a group of samples is being analyzed, simply attach the shutdown procedure to the end of the sequence. The Shutdown procedure allows the pumps to flow at a rate of 10 µl/min at 50% B with the detector lamps off.

### Short Term Shutdown

If the instrument will be shutdown for a few days or if running solvents that are too corrosive to leave in the pumps or column, run the shutdown procedure using wash solvent rather than solvents A & B.

### Long Term Shutdown

If the instrument is to be shutdown for more than a few days, a more extensive shutdown procedure may be necessary. First, flush the system with wash solvent for approximately 30 minutes at the appropriate flow rate for the column in use. Second, turn the pumps off and close the controller software. Turn the solvent select valves to the upward (Off) position so that no solvents are selected. Power off the instrument and PC. If Helium is still being supplied to the instrument, wash solvent will continue to be flushed through the pump head pistons.

If the instrument is to be shut down for an indefinite period of time or to be moved, then the system should also be depressurized and the Helium source shut off. To depressurize the system, first turn the Helium Valve to Off and choose Sparge/Vent on the Helium panel. Unscrew the Delrin plug on the cap insert of the wash bottle to allow the gas to escape. Retighten the Delrin plug once the gas has escaped.

# IV. System Maintenance

Proper maintenance of the instrument will help prolong the life of the MAGIC 2002™ and its components. Change solvents on a regular basis to help to keep contamination problems and/or particulate formation from occurring. Routinely check all solvent levels and fill the reservoirs as needed so as not to run the system dry. Follow the proper shutdown procedure to help prolong the life of the pump heads and detector lamps.

## Solvent Replacement

Whenever replacing solvents the system will need to be depressurized prior to opening the bottles. First turn all pump flows off and place the solvent select valves in the upward (Off) position. Turn the Helium to the Off position on the Helium panel and the Sparge/Vent valve to Sparge/Vent. Turn the main Helium supply off, and then vent the wash bottle by loosening the Delrin plug and allowing the gas to escape. The solvents can be replaced once the system has been completely depressurized. Once the solvents are replaced, then the system should be re-pressurized and checked for Helium leaks as discussed on page 22 under "Solvent Sparging and Detection of Helium leaks".

## Measuring Flow Rates

Flow measurement readings should be taken with the column in-line for consistent back pressure. Flow measurements are taken from the flow cell outlet tube for accurate readings. To take a flow measurement, the following items are needed: a union (Part Number 004-25027-00), the manual injection port (from port 1 of the injection valve), and a syringe with appropriate volume to see 2-5 minutes of flow.

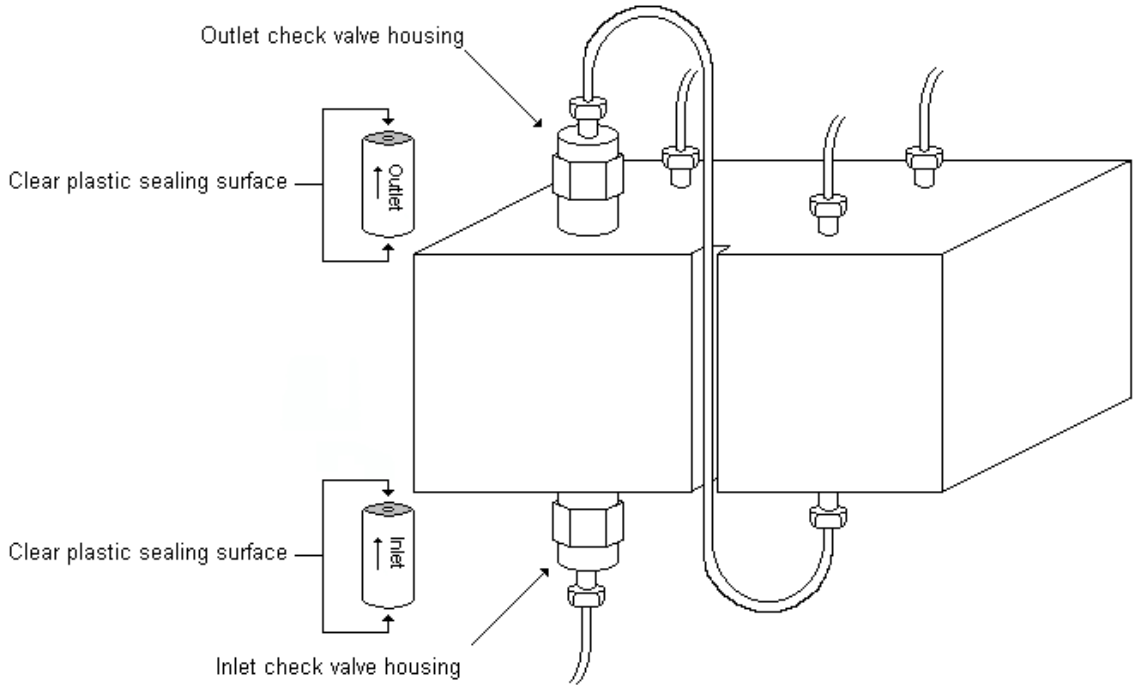
Prior to taking flow measurements, allow the system pressure to stabilize at the desired flow rate and initial gradient conditions. Once the system pressure is stable, disconnect the flow cell outlet tube from the stainless steel bulkhead union in the ceiling of the column compartment. Take the line, along with the long PEEK nut, and place it through the access port near the bulkhead union on the right panel of the instrument. Screw the union onto the PEEK nut that is now in the access port in the right panel. Remove the Manual Injection Port (*Refer to Figure 8, page 20)* from port 1 of the injection valve, and connect it to the union. A syringe can now be placed in the manual injection port to take flow measurements. For troubleshooting low or high flows, please refer to the Troubleshooting Guide at the end of this manual. Once the desired flow rate is achieved and/or confirmed, a standard can be analyzed to verify that the system is running properly.

## Replacing Check Valves

To replace an inlet and/or outlet check valve on a pump head, all pump flow and solvent flow must first be stopped. Remove the old check valve from the check valve housing by loosening the associated tubing from the housing with a ¼" open ended wrench and then loosen the housing itself with a ½" open ended wrench *(Refer to Figure 27, page 38)*. Note the orientation of the old check valve before it is removed. Dry the inside of the housing and then place the new check valve inside the housing with the proper orientation (The Outlet check valve arrow should be pointing away from the pump head. The Inlet check valve arrow should be pointing toward the pump head.) Reinstall the housing to the pump

head and the tubing to the housing. Do not over tighten. Once the new check valve is in place, follow the procedure outlined on page 24 under "Solvent Flow to the Pump Heads" and then purge the pumps to remove air as described on page 26 under "Purging Pumps to Remove Air".

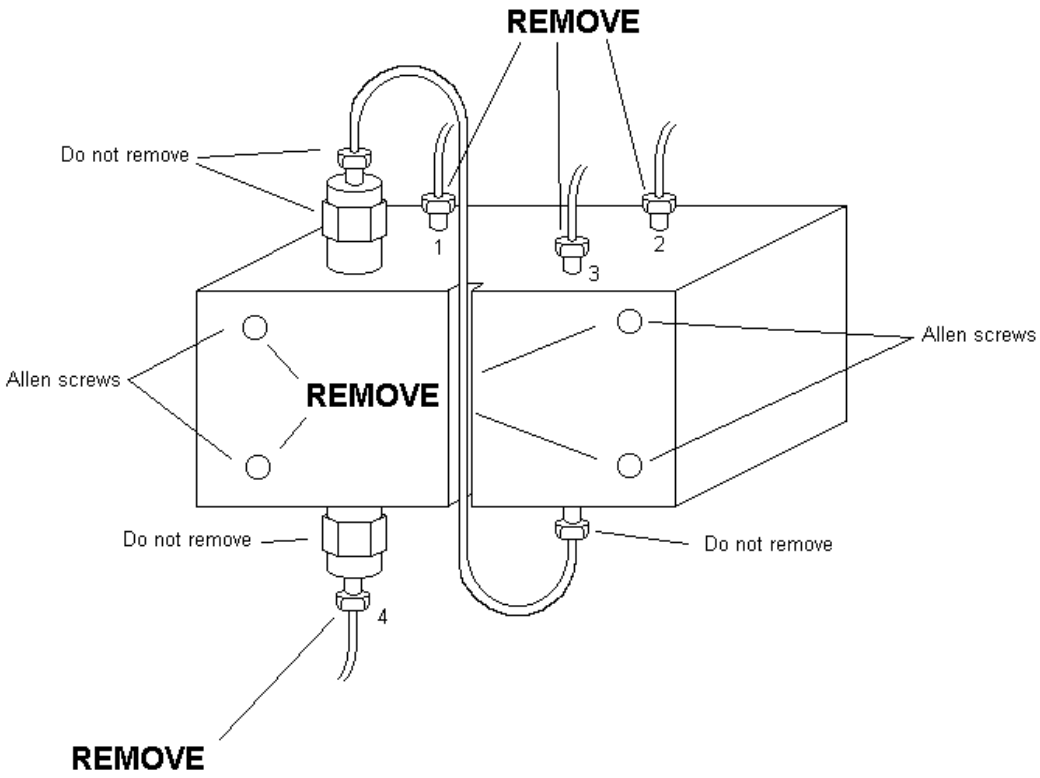
*Figure 27 Pump head*




## Replacing Pump heads

Prior to replacing a pump head, stop all pump flows and solvent flows, close EZChrom *Elite* software, and turn the power to the instrument off. Remove the tubing (*Refer to Figure 28, page 39)* by using a ¼" open ended wrench and then remove the pump head by unscrewing the four Allen screws. To install the new pump head, simply reattach the tubing and Allen screws. Once the new pump head is installed, turn the power to the instrument back on and allow the detector to re-initialize prior to re-opening the EZChrom *Elite* software. Verify solvent flow to the pump heads by following the procedure outlined on page 24 under "Solvent Flow to the Pump heads" and then purge the pumps to remove air as outlined on page 26 under "Purging Pumps to Remove Air".

*Figure 28 Pump head*




**Replacing Detector Lamps**

Prior to changing either detector lamp (D2 or Tungsten), close the controller software and power the instrument off. Remove the cover from the UV lamp compartment as shown on page 21 (*Refer to Figure 11, page 22)*. Disconnect the appropriate plug from the connector and remove the lamp by unscrewing the associated thumb screw(s). Replace the lamp, reconnect the plug to the connector and place the cover back on the UV lamp compartment. The instrument can now be powered on.

# V. Re-Installing The MAGIC 2002™

If an instrument has been moved and/or needs to be installed by someone other than a Michrom BioResources Inc. specialist, follow the procedure below for Helium connection and power connection. Refer to the section in this manual entitled "System Startup" (*Chapter 2; Section 1, page 23)* for guidelines to getting the system operational.

## Helium

Helium must be connected to the MAGIC 2002 for proper solvent delivery to the pumps. Helium is connected to the rear panel of the MAGIC using a 1/8 inch brass Swagelok nut and ferrule and 1/8 inch PEEK tubing (*Refer to Figure 3, page 13*). The other end of the tubing is connected to a Helium source regulator set between 10 and 200 PSI. ***DO NOT TURN HELIUM ON TO THE INSTRUMENT AT THIS TIME.***

Install solvent bottles A and B (filled with the appropriate solvents) in the front left door of the instrument (*Refer to Figure 2, page 12*). Install the wash solvent bottle (filled with the appropriate solvent) in the waste compartment located in the rear of the right side panel of the instrument (*Refer to Figure 4, page 14*). Turn the Helium On/Off valve located on the inside of the left front door to the Off position (*Refer to Figure 5, page 16*). Turn the "Pressurize Sparge/Vent" valve to the Pressurize position. Set Helium flow to the MAGIC 2002™ by opening the valve on the Helium source. Turn the Helium On/Off valve to the On position *(Refer to Figure 5, page 16)*. The filters in the bottom of the solvent bottles will begin to bubble (sparge) Helium into the solvents. This sparging will continue until the bottles are fully pressurized.

## Power

The MAGIC 2002™ can accept voltages of 100-120V AC and 220-240V AC. The power entrance to the MAGIC 2002 is located at the rear of the right side panel (*Refer to Figure 4, page 14*). This is where the power cord to the instrument is inserted and where the voltage selection switch is located. The MAGIC 2002™ is shipped with 115V AC as the default voltage selection, unless otherwise specified. If the instrument needs to be changed to 230V AC, remove the power cord to the instrument. Remove the voltage selection switch block using a small screwdriver. Change the fuses so that a 2.0A fuse is on the side labeled 110V-120V and a 2.5A fuse is on the side labeled 220V-240V. Insert the voltage selection block so that the white down arrow under the 220V-240V lines up with the white up arrow on the voltage selection socket on the MAGIC 2002™. Switch the voltage select switch to the 230V AC setting. The MAGIC 2002™ can run at 100V AC by keeping the original fuses in place at the 115V AC setting and simply changing the setting on the back of the UV/Vis Detector from 120V AC to 100V AC.

# CHAPTER 3

# OPTIONS

## I. Selecting Flow Range Kits for Optimum HPLC Conditions

Gradient delay volume, equilibration volume, sample injection volume and extra column volume can be optimized using the MAGIC 2002™ to achieve the true theoretical gains in sensitivity with microbore and capillary columns. The MAGIC 2002™ has been especially designed to be flexible for changing between analytical (100-5000µl/min), microbore (20-1000µl/min) and capillary flows (0.1-20µl/min) with the use of Flow Range Kits (Analytical, Microbore or Capillary). Each Flow Range Kit optimizes the system for that particular flow range to allow for the best sensitivity.

As shown in Table 2, the HPLC column diameter should be matched to the capacity of analytes (retained components of the injected sample) to insure optimum chromatography. Too large a sample load may cause excessive band spreading, saturation of detection and/or contamination of the column. However, too small a sample load may cause excessive analyte dilution, limited detection signal to noise and/or nonlinear recovery due to excess adsorptive surfaces. Sample injection volume can also be a rate-limiting step, since sample volumes greater than 10% of the column volume may contribute to band broadening (unless specialized loading techniques are employed).

*Table 2 HPLC Column Capacity & Flow Conditions*

| Column ID (µ) | 5cm Bed Vol (µl) | Analyte Capacity (grams) | Optimum Flow Rate (µl/min) | Flow Rate Range (µl/min) | Optimum MAGIC Flow Range Kit |
|---|---|---|---|---|---|
| 50 | 0.1 | $<10^{-12}$ | 0.10 | 0.05-0.50 | Capillary |
| 100 | 0.4 | $10^{-10}$ - $10^{-14}$ | 0.50 | 0.25-2.50 | Capillary |
| 200 | 1.6 | $10^{-9}$ - $10^{-13}$ | 2.00 | 1.00-10.0 | Capillary |
| 300 | 3.6 | $10^{-8}$ - $10^{-12}$ | 5.00 | 2.00-20.0 | Capillary |
| 500 | 10 | $10^{-7}$ - $10^{-11}$ | 12.0 | 5.00-50.0 | Microbore |
| 1000 | 40 | $10^{-6}$ - $10^{-10}$ | 50.0 | 20.0-200 | Microbore |
| 2000 | 160 | $10^{-5}$ - $10^{-9}$ | 200 | 100-1000 | Microbore |
| 3000 | 360 | $10^{-4}$ - $10^{-8}$ | 500 | 200-2000 | Analytical |
| 4600 | 800 | $10^{-3}$ -$10^{-7}$ | 1000 | 500-5000 | Analytical |

With the analytical or microbore Flow Range Kits installed on the MAGIC 2002™, no special procedures are required to achieve optimum performance.

## II. Capillary Flows

The Capillary Gradient Flow Range Kit includes microbore pump heads, a 100 µl mixer cartridge, a pre-column splitter, a capillary flow cell assembly, and a post column splitter. The capillary system is designed to run the microbore pump heads at optimal flows while delivering the desired capillary flows by utilizing a specially designed pre-column splitter.

*Figure 29 MAGIC 2002™ as Capillary Unit*

## The MAGIC Variable Splitter™

The MAGIC Variable Splitter™ (MVS™) is designed for use with the MAGIC 2002™ (*Refer to Figure 30*). The MAGIC Variable Splitter™ utilizes a unique approach for capillary HPLC. The MVS™ consists of an off-line variable splitter box, a splitter tee, and capillary FSLP (Fused Silica Lined PEEK) tubing. The variable splitter box contains five different packed bed restrictors which vary in pressure drop in the same way a packed bed HPLC column does. This provides a reproducible split ratio allowing a consistent flow rate across the capillary column. Unlike inline splitters, the MVS™ was designed to add no extra system volume, thus allowing fast gradient separations at capillary flow rates.

*Figure 30 MAGIC Variable Splitter™*


Optimum Input Flow Range: 50-100 µl/min
Effective Input Flow Range: 20-200 µl/min
Capillary Column Output Flow Range: 0.2-25µl /min
Optimum Split Flow Range: 1:1 to 1:100
Effective Back pressure Range: 50-4500 PSI
Flow Rate Reproducibility: +/- 0.1 %

## Capillary Flow Range Kit Setup

If the MAGIC 2002™ was purchased as a capillary unit, the system will come with the Capillary Flow Range Kit installed. However, if the MAGIC 2002™ is set up as a Microbore unit, the following procedure should be followed:

1. Turn flows to the pumps off and close the solvent select valves.
2. Remove both loops from the injector valve, the mixer to valve tube, and the column inlet tube.
3. Connect the splitter tee to the injection valve port 4 using the Lite-Touch fitting on the 10 cm Fused Silica Lined PEEK tubing (FSLP).
4. Connect the splitter tee to the mixer output using the Lite-Touch fitting at the end of the 5 cm stainless steel tubing.
5. Thread the third line from the splitter tee (with micro filter in place) out through the lower access port in the right side panel, and connect to port R3 on the MAGIC Variable Splitter™ .
6. Connect the Teflon waste line to port W3 on the MAGIC Variable Splitter™ and place the outlet of the waste line into the MAGIC 2002™ waste container.
7. Connect the sample PEEK loop or capillary trap/loop of choice between ports 3 and 10 on the injection valve.
8. Connect the bypass PEEK loop between ports 5 and 8 on the injection valve.
9. Connect the column inlet tube to port 9 on the injection valve.
10. Close the controller software and power the unit off.
11. Install the Capillary Flow Cell Assembly in place of the Microbore Flow Cell Assembly.
12. Turn the power to the instrument back on (wait 2-3 minutes for detector to reinitialize) and then restart the controller software.

## Checking for Leaks

After installing a column, check the new setup for leaks. To do this, turn the solvent select valves on and start the pumps flowing at 100 uL/min at 50 % B. Check all fittings around the injection valve, the mixer, and the MAGIC Variable Splitter™ for leaks and confirm flow through the column inlet tube.

## Column Installation

With the capillary system, it is especially important to minimize the introduction of air to the flow cell. Once flow through the column inlet tube is confirmed, allow the system to run for approximately 3-5 minutes. Turn the pump flow off and dry the end of the tube. Connect the column to the column inlet tube. Refer to the Column Test Certificate for an approximation of flow parameters to achieve the desired flow rate. Choose the MAGIC Variable Splitter™ level and set the flow rate in the control software according to the test certificate. Change the

waste line on the MAGIC Variable Splitter™ to the W position directly opposite the R setting. If no test certificate is available, a good starting point is R3 with a flow rate of 100 µl/min. Confirm flow through the column prior to connecting it to the flow cell inlet tube. Once the system pressure has stabilized, check the flow cell outlet tube to ensure some liquid is flowing through the column and flow cell. If no measurable flow is obtained in 15-20 minutes, there may be a leak or blockage in the system. Check the fittings around the injector, from the column to the flow cell, and the flow cell outlet fittings.

**Measuring and Adjusting Flows**

Measure the flow rate as described on page 37 under "Measuring Flow Rates", and adjust the pump flow rate and/or the restriction as needed to achieve the desired capillary flow rate. When it is necessary to switch from one splitter level to another, turn pump flow off and allow the column to depressurize to less than 100 PSI.

If the measured flow rate is more than the desired capillary flow rate, simply decrease the pump flow rate. If it is necessary to decrease the pump flow significantly below 50 µl/min, then it is best to move the tubing going into the Variable Splitter Box to an R value setting that provides less restriction. This will allow the pumps to run under optimal conditions of 50-100 µl/min, yet deliver the appropriate flow through the column.

If the measured flow rate is less than the desired capillary flow rate, move the tubing going into the MAGIC Variable Splitter™ to another R value setting of higher value. Change the pump flow rate by factors of 2 to achieve the desired capillary flow through the column (another change in restriction may also be necessary). Keep in mind that the optimal pump flow conditions are 50-100 µl/min.

**Running Samples in the Split-Flow Mode**

Once the desired flow rate has been achieved, set the solvent composition to the initial conditions of the desired method. Ensure the method contains the proper pump flow rate prior to running a sample. Follow the operation procedures as outlined in Chapter 2 under Operating the MAGIC 2002™.

# III. Auto sampler

Although a wide variety of auto samplers can be utilized along with the MAGIC 2002™, Michrom BioResources, Inc. offers an auto sampler optimized for use with the MAGIC 2002™. Please refer to the manual that comes with each auto sampler for detailed information on operation, features, and options.

## Connecting a Michrom BioResources, Inc. Auto sampler to the MAGIC 2002™

The auto sampler will need to be wired to the MAGIC 2002™. Please refer to the wiring diagram (*Figure 31*) for connection to External Start Out and External Trigger Input on the rear panel of the instrument.

The auto sampler can be plumbed to the injection valve of the MAGIC 2002™ several different ways. Refer to the plumbing diagram (*Figure 32*) for the standard setup.

*Figure 31 Auto Sampler Wiring*

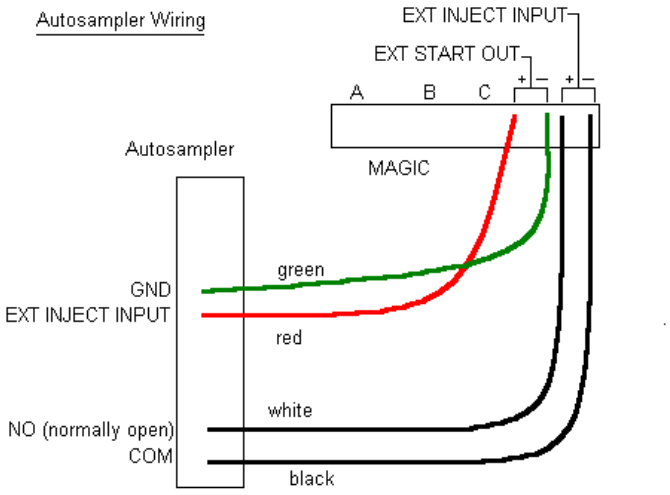
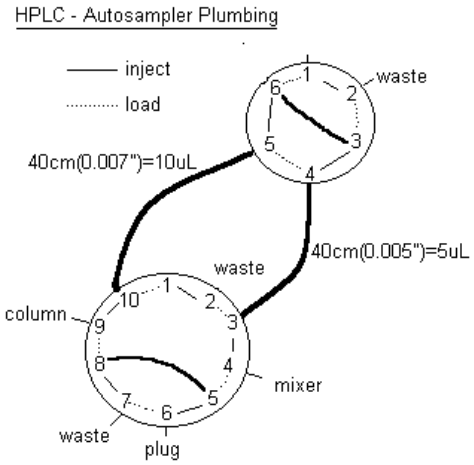



*Figure 32 Auto Sampler Plumbing*



### Operating with a Michrom BioResources, Inc. Auto sampler

Whenever an auto sampler will be used to trigger the MAGIC 2002™, it is necessary to set the trigger in the control software to "External". Program the auto sampler to run according to the desired parameters with the correct number of injections.

## IV. Trapping Options

Michrom BioResources, Inc. has designed trap cartridges that allow automated sample preparation (concentration, desalting, buffer exchange and detergent removal) prior to analysis by the MAGIC 2002™.

### Configuration on the MAGIC™

The traps are designed along with a trap holder to take the place of the sample loop (port 10 and port 3) on the injection valve.

### Using a Trap Cartridge

To achieve the best results using a trap, some sample handling requirements are necessary. The sample should be loaded in the solution which ensures solubility of all the components of interest (Solvent A), yet has very low chromatographic mobility. The trap should be washed with a solution similar to the sample loading solution in sufficient volume to remove all of the salts and other potential interfering components without eluting the analytes of interest. The trap should be back flushed with a gradient to strip the components of interest from the trap in very concentrated bands.

For detailed information on various traps and their uses, please refer to the specific trap literature available from Michrom BioResources, Inc.

## V. Fraction Collector

Although a wide variety of fraction collectors can be used with the MAGIC 2002™ HPLC System, Michrom BioResources, Inc. offers a "Micro Fraction Collection System" optimized for use with the MAGIC 2002™. Please refer to the manual that comes with the fraction collector for information on operation features and options. *(Refer to Figure 33-35, page 48-50).*

*Figure 33  Fraction Collector Wiring Step 1*

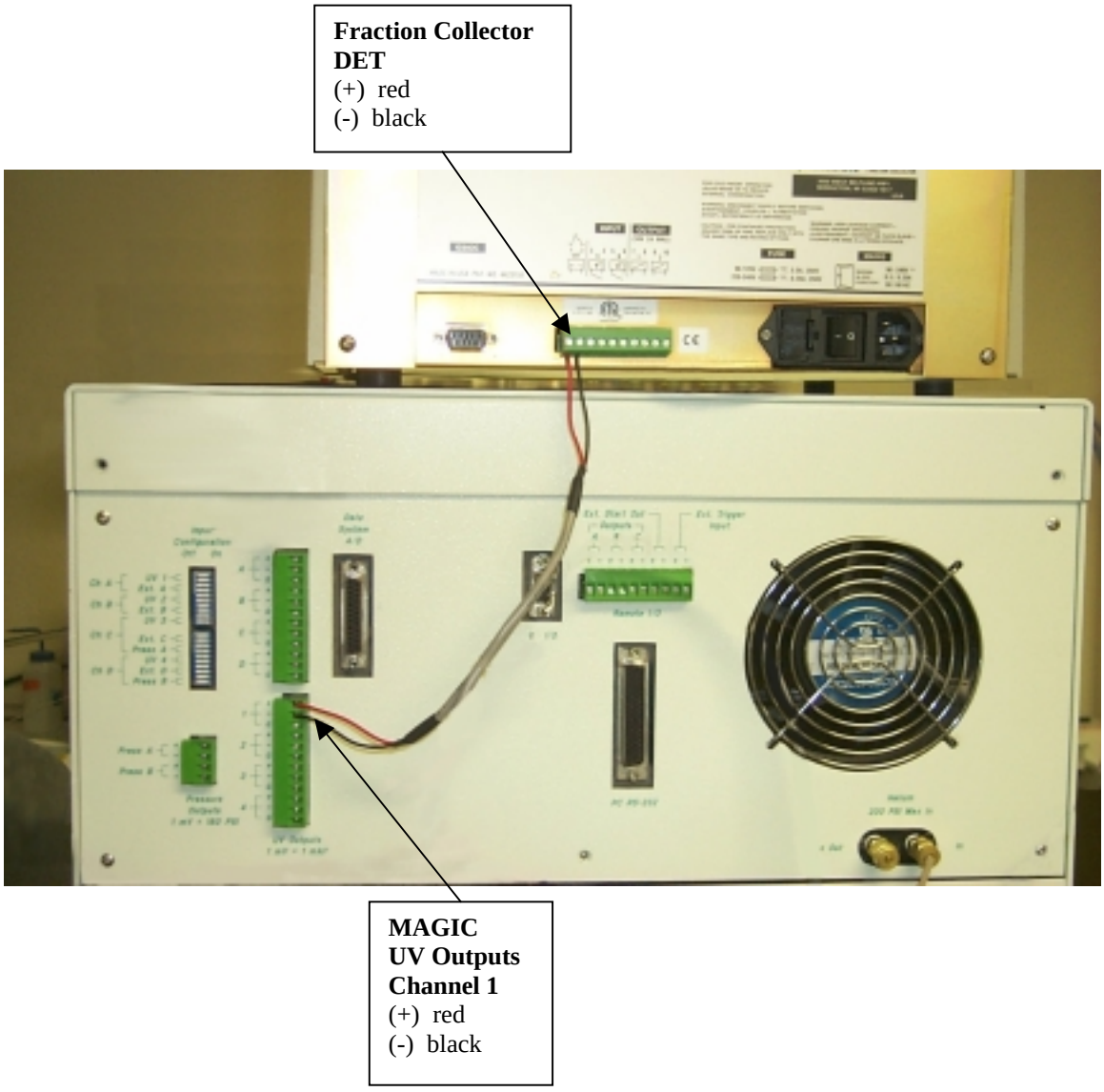


*Figure 34  Fraction Collector Wiring Step 2*

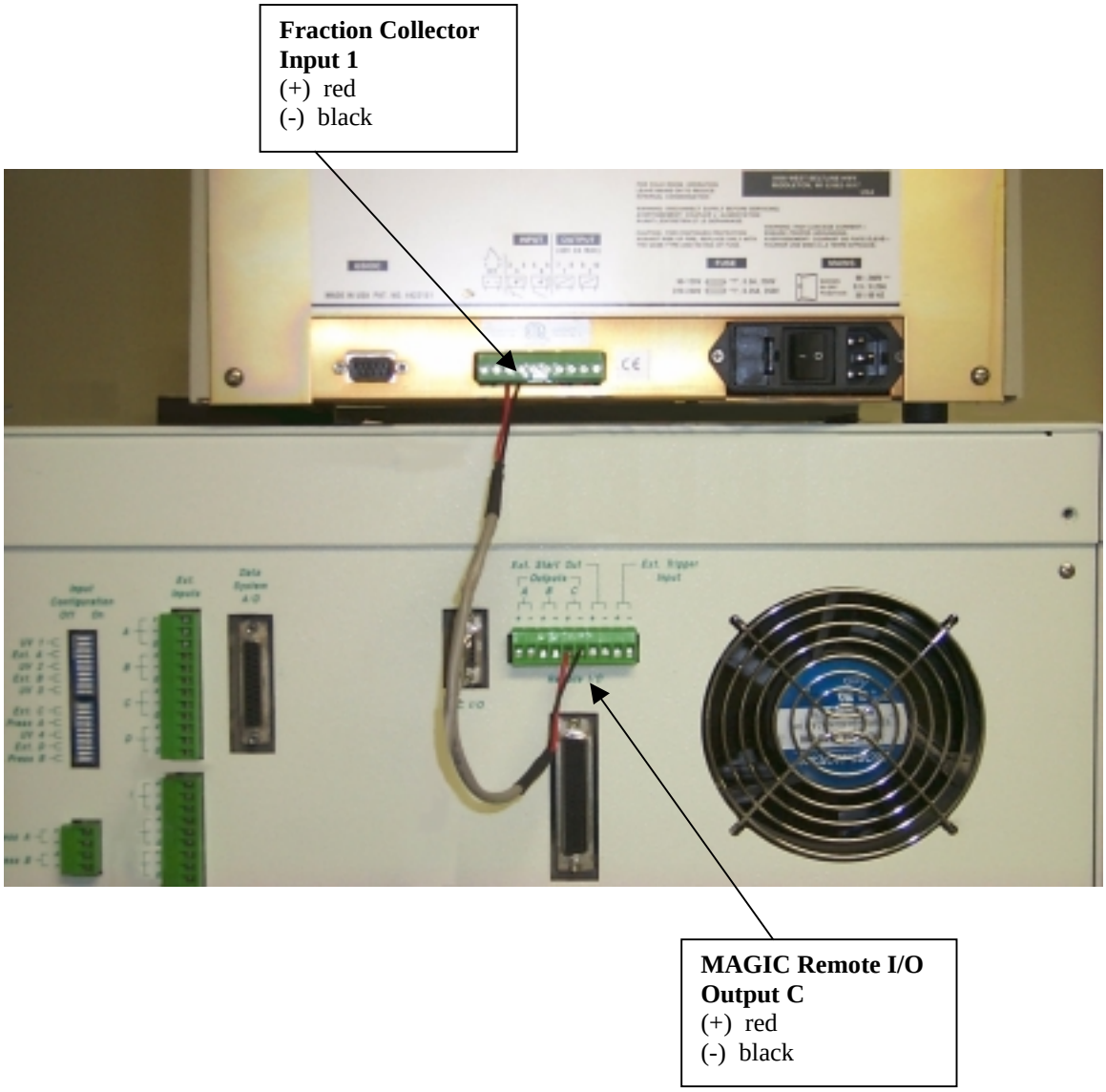
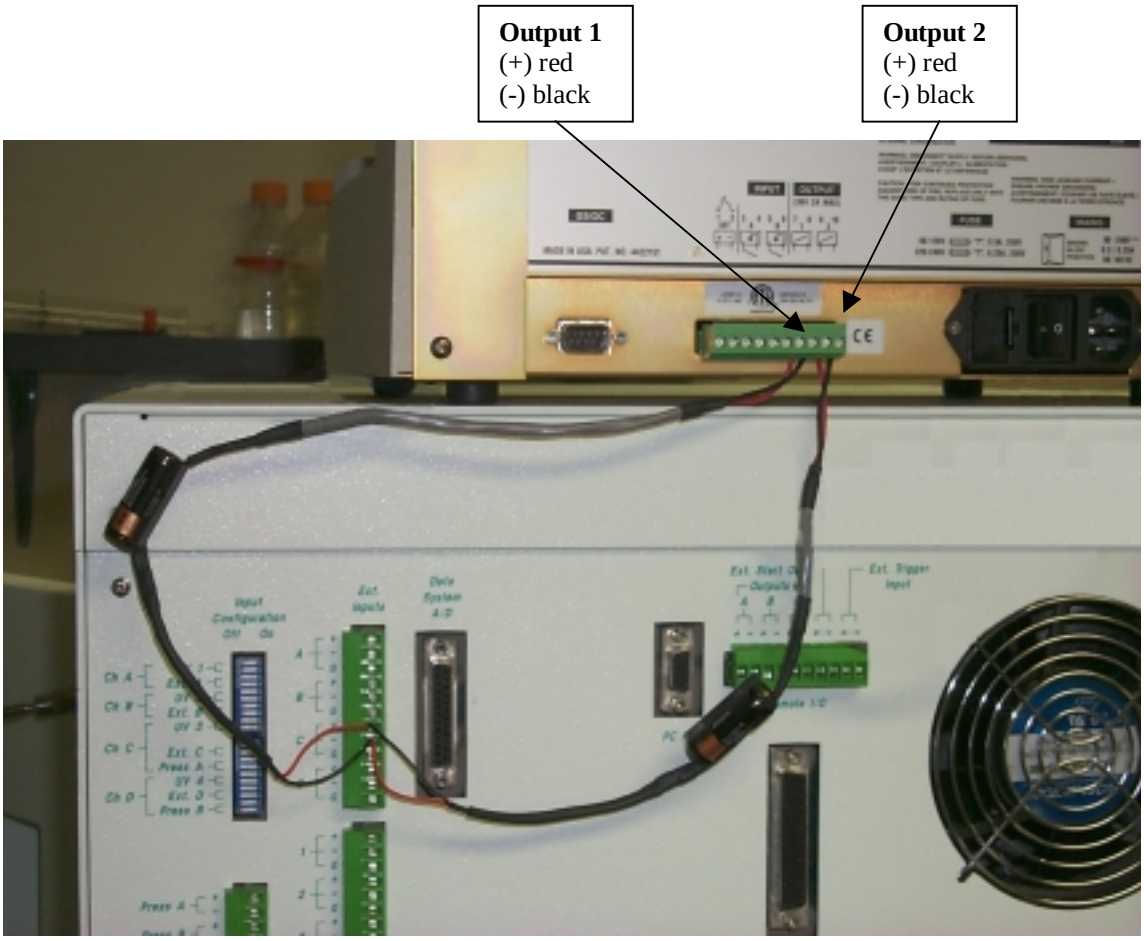


*Figure 35  Fraction Collector Wiring Step 3*




**MAGIC External Inputs** **Fraction Collector Outputs**

**C (+)** → **Output 1 (+) red**
**Output 2 (-) black**

**C (-)** → **Output 1 (-) black**
**Output 2 (+) red**

This wiring configuration will show downward tick marks at the beginning of each collected peak and upward tick marks at the end of each collected peak in the EZChrom *Elite* data acquisition screen.

# Appendix

## I. Troubleshooting Guide

| SYMPTOM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| No power to instrument. | Power cord is not properly plugged into instrument. | Plug power cord into instrument. |
| | Power entrance fuses are blown. | Replace fuses with known good 5 A and 3.5 A fuses. |
| Power to instrument but no lights on pump panels. | Pump fuses blown at pump power entrance. | Replace fuses with known good 500 mA fuses. |
| Computer does not communicate with the instrument. | Communication cables are not connected from the computer to the instrument. | Plug all cables into the proper ports on the back of the computer and on the back of the instrument. |
| EZ Chrom Data System only operates in the Demo Mode. | The EZ Chrom license key is not plugged into the printer port on the back of the computer. | Plug the EZ Chrom license key into the printer port in the back of the computer. |
| The Magic Control software does not communicate with the instrument. | The instrument needs to be started and internal diagnostics completed before communication is attempted through the control software. | Close the instrument control software and power off the instrument. Power the instrument up and let it warm up for several minutes before attempting to communicate with it through the control software. |
| One or both solvent bottles continually sparge or bubble. | There is a leak in the Helium system internal to the instrument. | Check for Helium leaks around the bottle caps and bottle cap insert fittings. Delrin plug in Wash bottle cap insert is loose or missing. |
| | There is a leak in the Helium system external to the instrument. | Check for Helium leaks around the tank and all external fittings to the instrument. Tighten fittings as necessary. |
| No solvent to pump head inlet. | No solvent in solvent bottles. | Fill solvent bottles with appropriate solvent. |
| | No Helium pressure to solvent bottles. | Make sure Helium tank valve is open and appropriate Helium pressure is set at the tank regulator. |
| | He On/Off valve on inside left door of instrument is in the “Off” position. | Turn the He On/Off valve to the “On” position. |

| SYMPTOM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| No solvent to pump head inlet (Continued). | Plugged tubing in the Helium system. | Isolate the plugged tubing and clear the blockage or replace the tube. |
| No pressure with flow set in the Magic control software. | One or both purge valves are open to the waste compartment. | Close the purge valve by turning clockwise until tight. |
| | No analytical column is installed with column inlet tubing attached. | Install analytical column and attach column inlet tubing to head of column. |
| | There is a leak at a fitting on a pump head, purge valve or the 10-port valve. | Shut flow off to pumps via the control software. Turn solvent selection valves to the vertical or "Off" position. Remove the fitting and thoroughly dry ferrule and ferrule seat using a Kimwipe. Retighten fitting. |
| | Pump head check valve(s) failed. | Replace check valve(s) with new or rebuilt check valve(s). |
| | Pump head seals are worn or scored. | Run pumps isocratically in wash solvent at 50 µl/min, 5% pump B, for 30 minutes. Save pressure data. Now run pumps isocratically in Wash solvent at 50 µl/min, 95% pump B, for 30 minutes. Save pressure data. Fax data to Michrom BioResources, Inc. for diagnosis by technical support personnel. |
| | Pump head piston(s) are worn or scored. | Operate pumps isocratically in wash solvent at 50 µl/min, 5% pump B, for 30 minutes. Save pressure data. Now run pumps isocratically in wash solvent at 50 µl/min, 95% pump B, for 30 minutes. Save pressure data. Fax data to Michrom BioResources, Inc. for diagnosis by technical support personnel. |

| SYMPTOM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| No pressure with flow set in the Magic control software (Continued). | Air bubble trapped in pump head. | Disconnect column inlet tubing. Set the pumps on purge via the software and open and close both purge valves on pumps to release trapped air bubbles. |
| Wavy or choppy UV baseline trace. | Flow cell leak. | Remove column, photodiode and flow cell assembly. Thoroughly dry quartz flow cell and reinstall into flow cell holder and bracket. Ensure proper alignment of flow cell in bracket before reinstalling assembly in column compartment. |
| | Air bubble trapped in flow cell | Run wash solvent at twice normal flow rate for about 30 minutes. Re-equilibrate at initial run conditions. |
| | Lack of efficient mixing. | Check to ensure that a mixer cartridge of sufficient volume is installed. in the mixer cartridge holder. |
| No peaks are observed when a sample is run. | The 10-port valve is in the **inject** position when the sample is loaded from the syringe. | Make sure that the 10-port valve is in the **load** position when the sample is loaded from the syringe. |
| | The sample was prepared in an inappropriate solvent for the application. | Prior to loading the sample into the sample loop of the instrument, be sure the sample is in solvent which is less than 5% organic in composition. |
| | The $D_2$ lamp has expired. | Replace the $D_2$ lamp with a new one. |
| | The active procedure does not specify the lamp being ignited and a wavelength selected. | Verify that the detector is turned on and a wavelength selected in the active procedure. |
| | The programmed gradient does not call for sufficient B (organic) solvent to be delivered to enhance peak elution. | Program the gradient to deliver at least 65% B solvent (~ 90% organic) to sufficiently enhance peak elution. |

| SYMPTOM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|
| No peaks are observed when a sample is run (Continued). | The sample loop contains a high percentage of organic solvent. This has caused the peaks to elute in the solvent front. | Flush the sample loop with a low percentage of organic solvent (~ 2%) with the valve in the **load** position. |
| Irreproducible retention times. | There is a leak at a fitting on a pump head, purge valve or the 10-port valve. | Shut flow off to pumps via the control software. Turn solvent selection valves to the vertical or "Off" position. Remove the fitting and thoroughly dry ferrule and ferrule seat using a Kimwipe. |
| | Pump head check valve(s) failed. | Replace check valve(s) with new or rebuilt check valve(s). |
| | Pump head seals are worn or scored. | Run pumps isocratically in Wash solvent at 50 µl/min, 5% pump B, for 30 minutes. Save pressure data. Now run pumps isocratically in Wash solvent at 50 µl/min, 95% pump B, for 30 minutes. Save pressure data. Fax data to Michrom BioResources, Inc. for diagnosis. |
| | | Run pumps isocratically in Wash solvent at 50 µl/min, 5% pump B, for 30 minutes. Save pressure data. Now run pumps isocratically in Wash solvent at 50 µl/min, 95% pump B, for 30 minutes. Save pressure data. Fax data to Michrom BioResources, Inc. for diagnosis by technical support personnel. |
| | Air bubble trapped in pump head. | Disconnect column inlet tubing. Set the pumps on purge via the software and open and close both purge valves on pumps to release trapped air bubbles. |

# II. Configuring the MAGIC 2002™ Instrument for use with Data Acquisition

After installation of the EZChrom *Elite* and MAGIC 2002™ software, it is necessary to configure the computer hardware. To configure the MAGIC 2002™ hardware, first open EZChrom *Elite* by clicking on **Start**, going to **Programs**, going to **Chromatography** and selecting **EZChrom *Elite***. Figures 36 through 50 present step by step instructions for configuring the computer hardware for instrument control and data acquisition.

*Figure 36 EZChrom Elite Client/Server Screen*

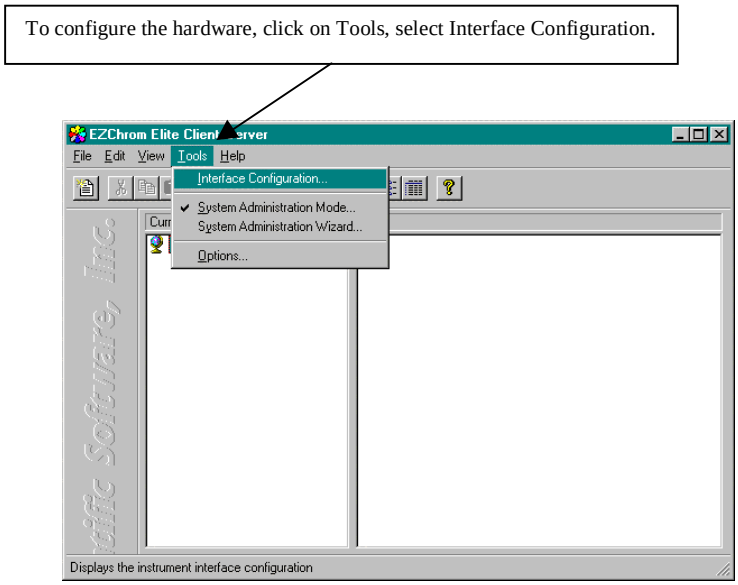



*Figure 37 Interface Configuration Screen*

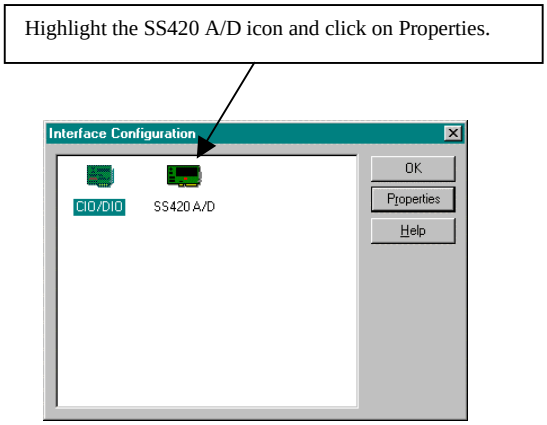
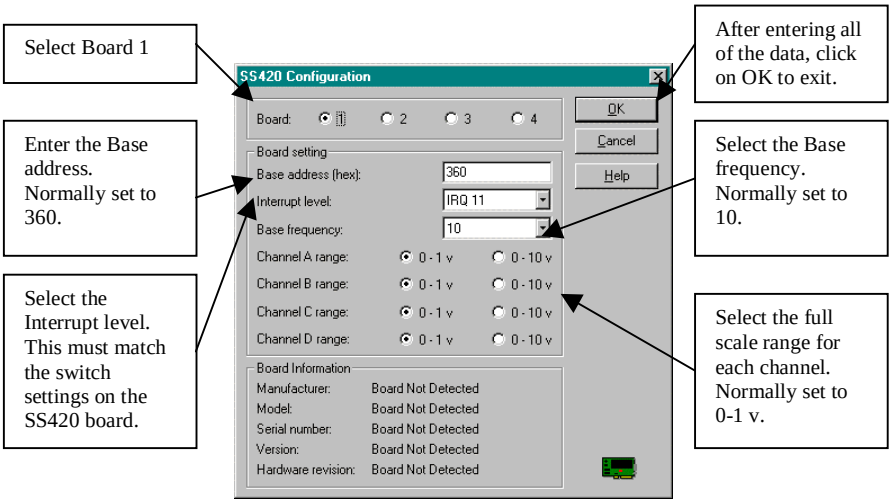


*Figure 38 SS420 Configuration Screen*




After setting the Interface configuration the computer must be restarted for the new hardware settings to take effect. When the computer restarts, click on **Start**, go to **Programs**, go to **Chromatography** and select **EZChrom *Elite***.

*Figure 39 EZChrom Elite Client/Server Screen*

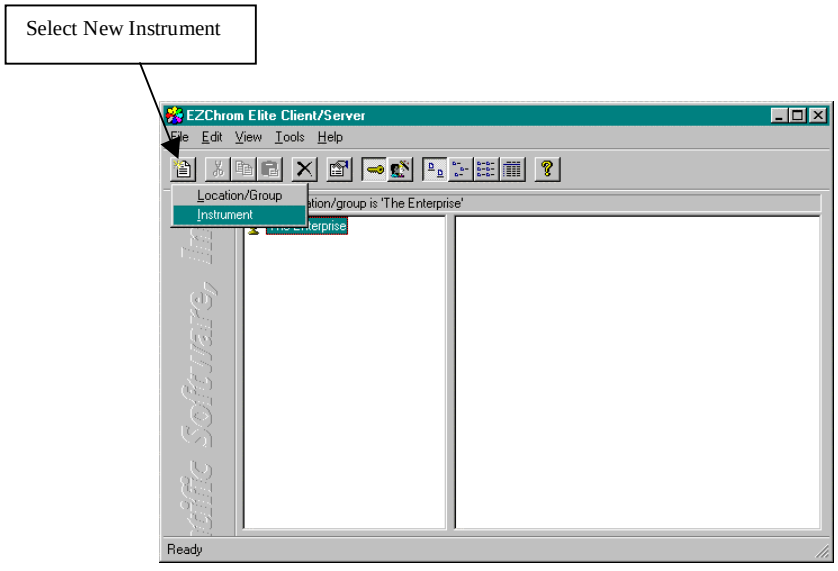


*Figure 40 EZChrom Elite Client/Server Screen*

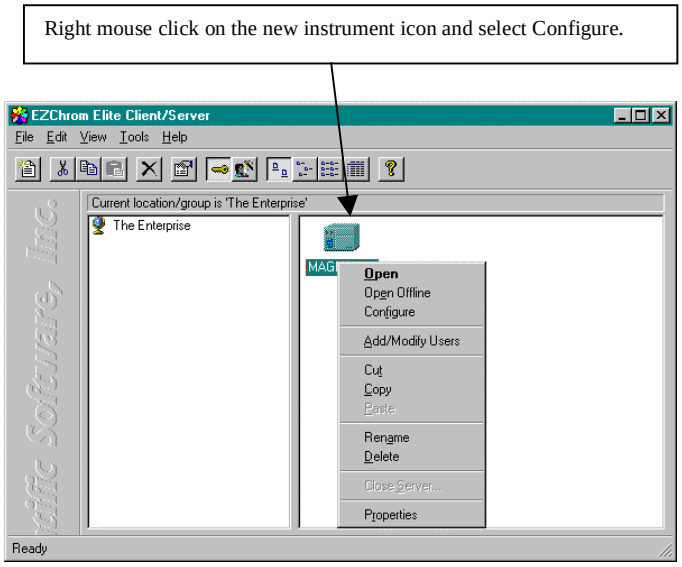



*Figure 41 Instrument Configuration Screen*

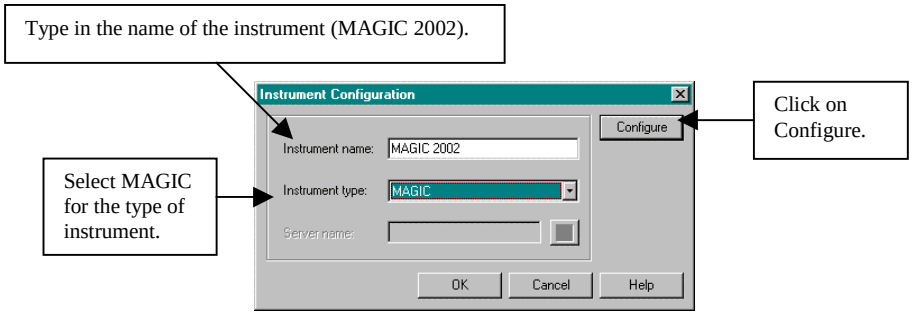
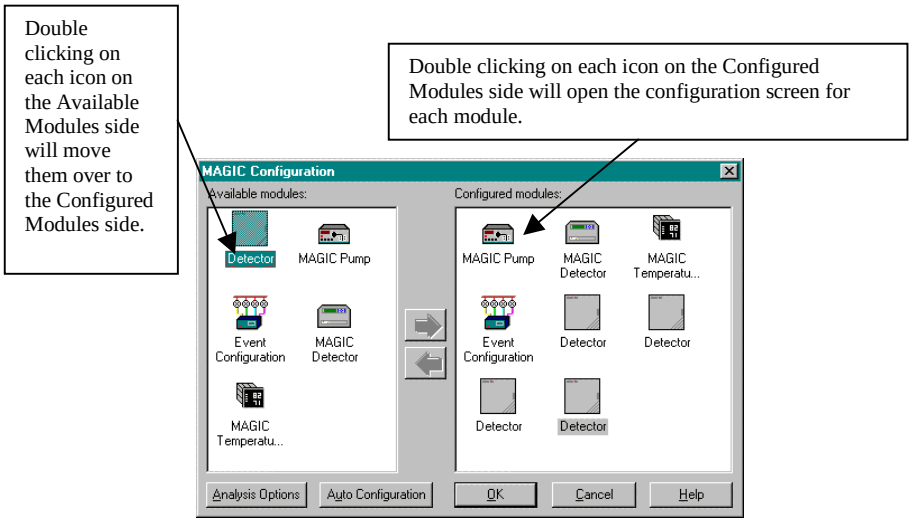
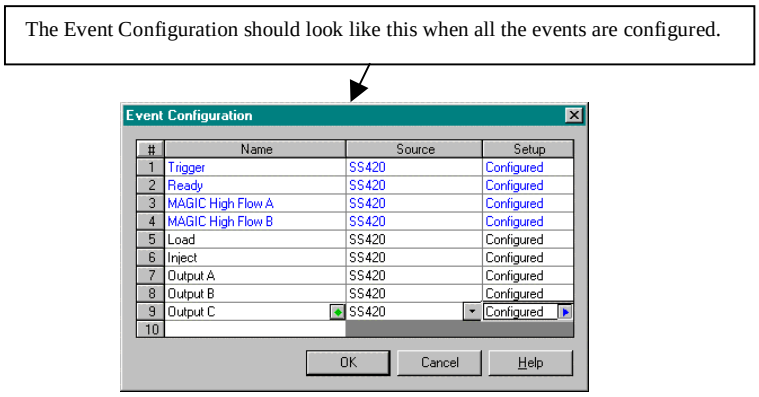


*Figure 42 MAGIC Configuration Screen*




Move all of the Modules from the Available module side to the Configured module side. Move three additional detector modules as shown above (one for each of the four channels of A/D input available with the MAGIC Elite data system.) Configure the Event Configuration first (*Refer to Figure 43 and Table 3, pages 58-59 )*, and then continue on until all the modules have been properly configured as shown on the following pages (*Refer to Figures 44-50, pages 59-62)* .

*Figure 43 Event Configuration Screen*



*The following table lists the Events by Name, the Source and the Setup for each event*

*Table 3 Event Configuration*

.

| Name | Source | Setup | | |
|---|---|---|---|---|
| | | Board | Input | Triggered State |
| Trigger | SS420 | 1 | In 1 | Closed |
| Ready | SS420 | 1 | Out 5 | Closed |
| MAGIC High Flow A | SS420 | 1 | Out 1 | Closed |
| MAGIC High Flow B | SS420 | 1 | Out 2 | Closed |
| Load (must be typed in) | SS420 | 1 | Out 3 | |
| Inject (must be typed in) | SS420 | 1 | Out 4 | |
| Output A (must be typed in) | SS420 | 1 | Out 6 | |
| Output B (must be typed in) | SS420 | 1 | Out 7 | |
| Output C (must be typed in) | SS420 | 1 | Out 8 | |

*Figure 44 MAGIC Pump Configuration Screen*

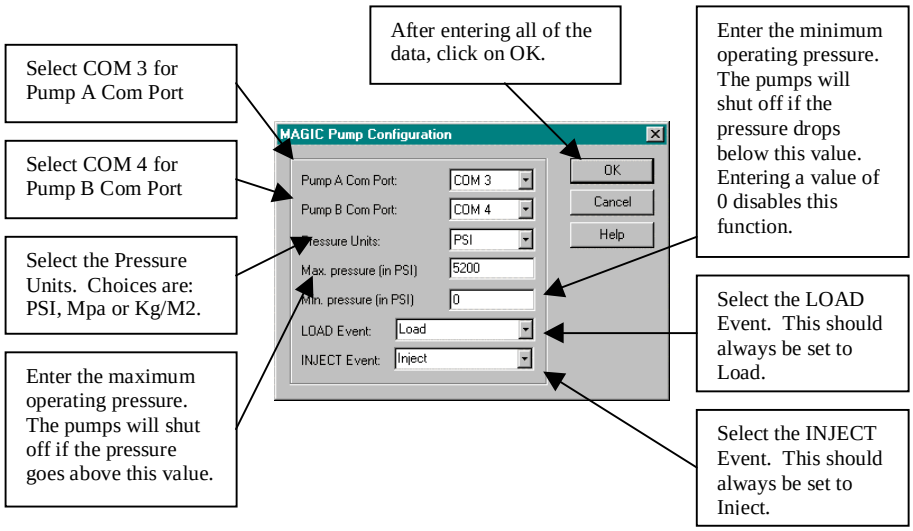


*Figure 45 MAGIC Detector Configuration Screen*

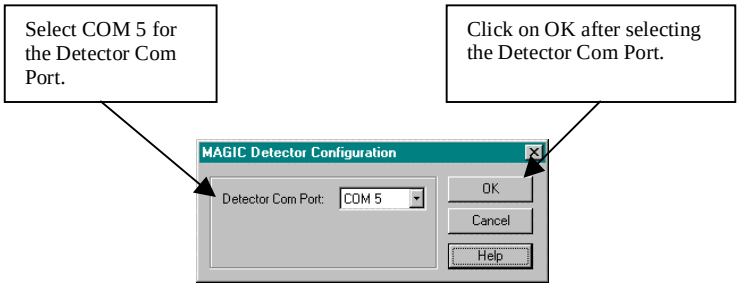



*Figure 46 MAGIC Temperature Controller Configuration Screen*

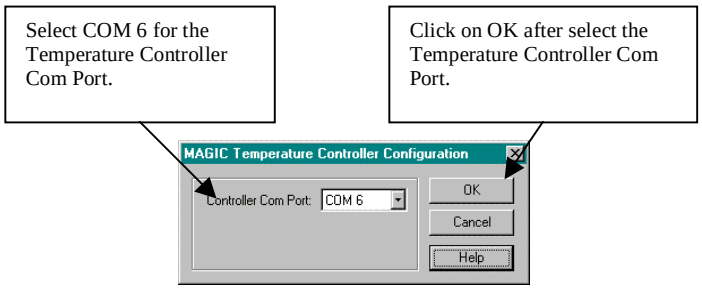



*Figure 47 Detector Configuration Screen (UV 1)*

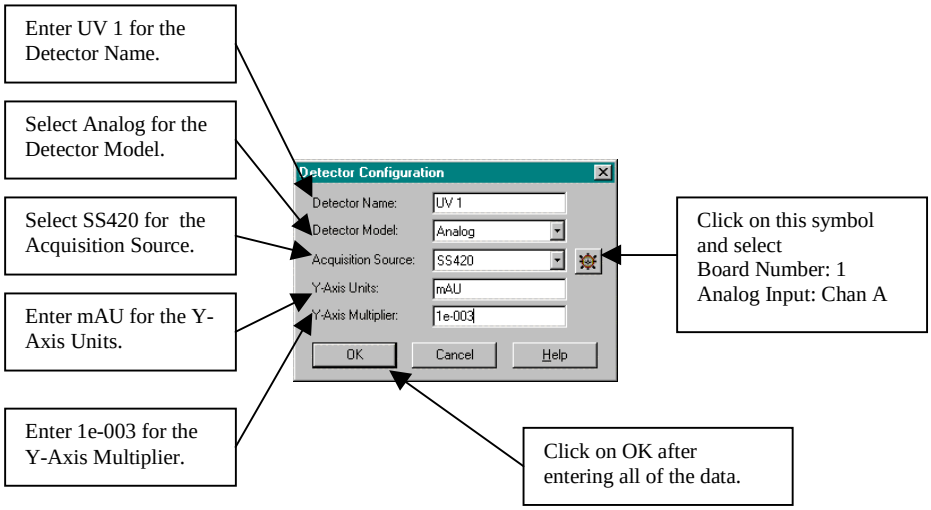


*Figure 48 Detector Configuration Screen (UV 2)*

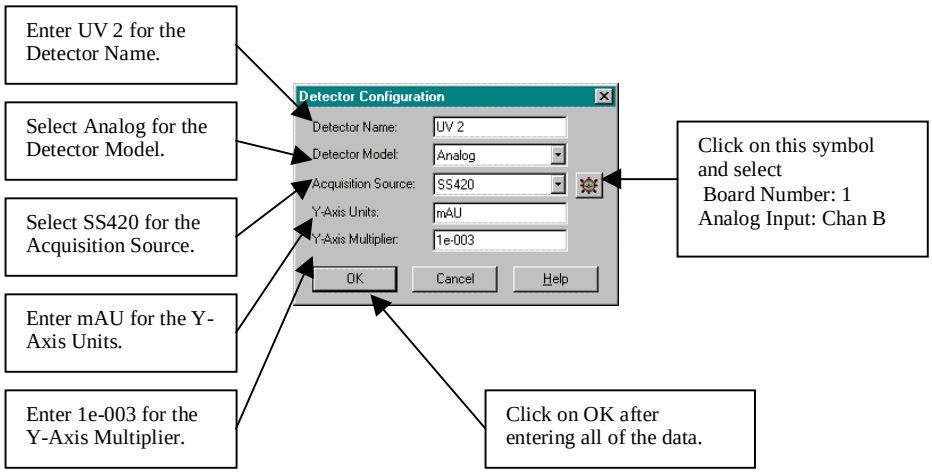



*Figure 49 Detector Configuration Screen (Fraction Collector)*

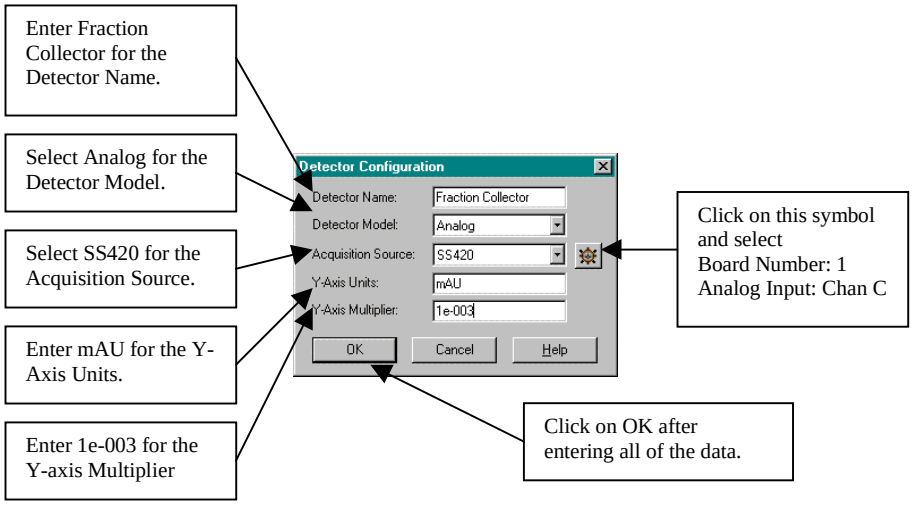
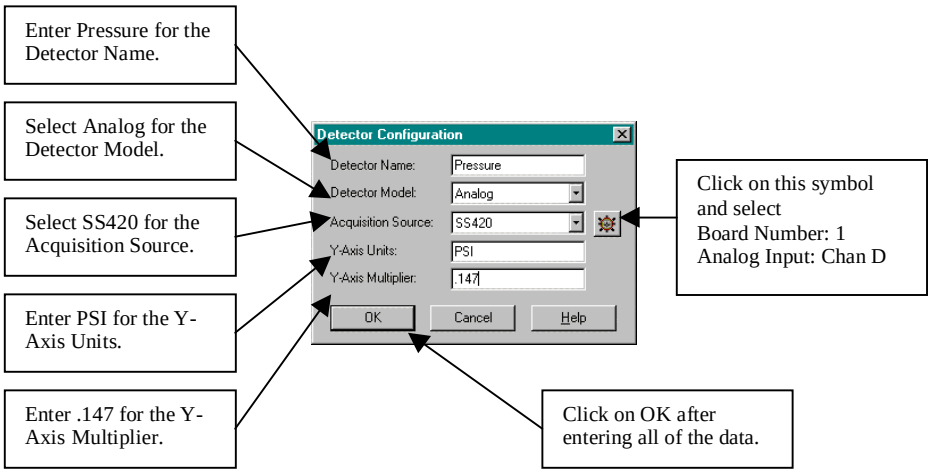


*Figure 50 Detector Configuration Screen (Pressure)*




After all eight modules have been configured, click **OK** on the MAGIC Configuration screen to save these configurations and return to the MAGIC EZChrom Elite Client/Server screen. Double-click on the MAGIC 2002™ icon to open the MAGIC software.

# III. Configuring the MAGIC 2002™ Instrument for Control Only

After installation of the EZChrom *Elite* and MAGIC 2002™ software, it is necessary to configure the computer hardware. To configure the MAGIC 2002™ hardware, first open EZChrom *Elite* by clicking on **Start**, going to **Programs**, going to **Chromatography** and selecting **EZChrom *Elite***. Figures 51 through 61 present step by step instructions for configuring the computer for instrument control.

*Figure 51 EZChrom Elite Client/Server Screen*

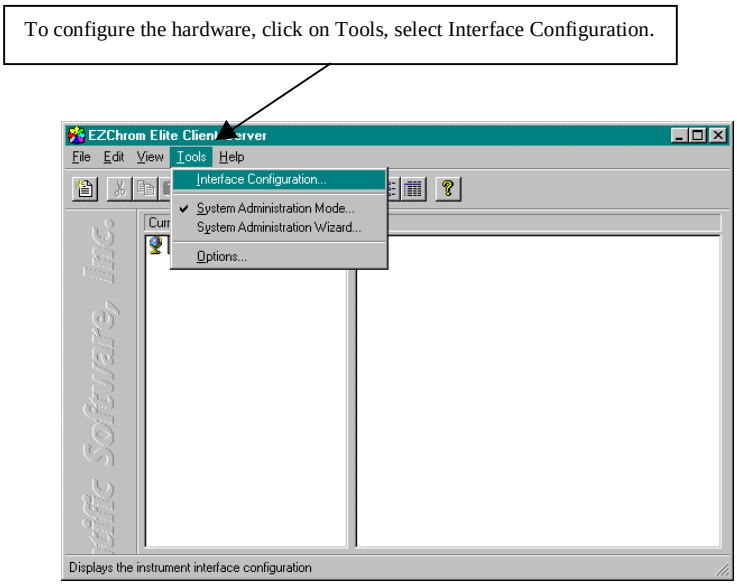



*Figure 52 Interface Configuration Screen*

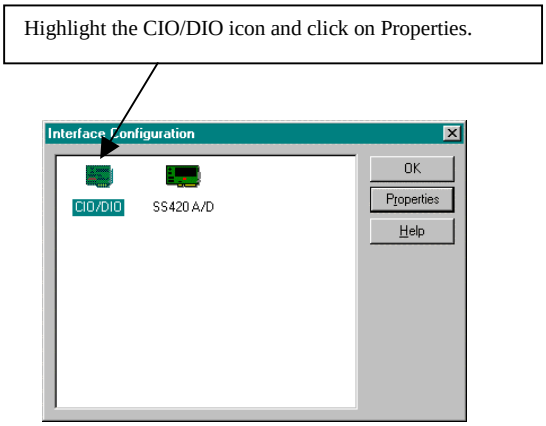
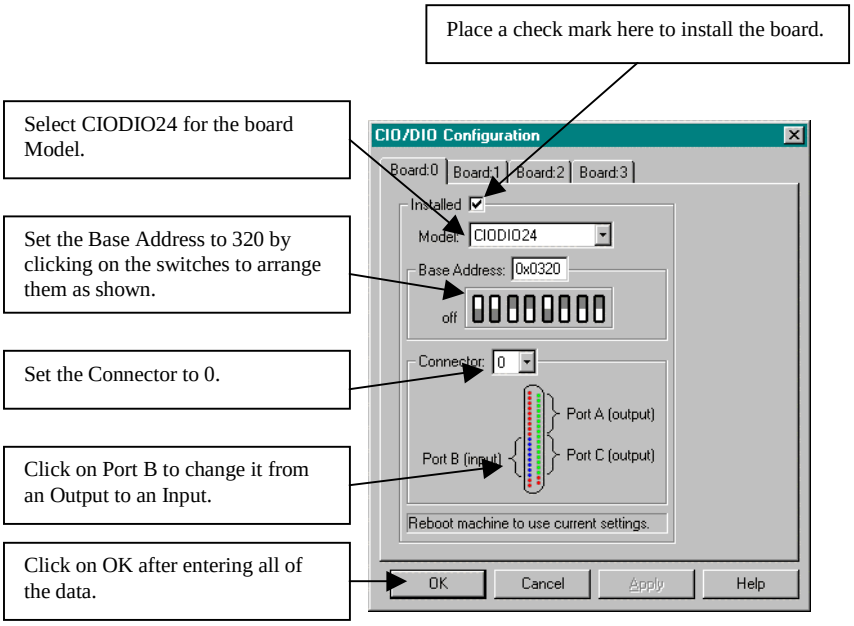


*Figure 53 CIO/DIO Configuration Screen*




After setting the Interface configuration, the computer must be restarted for the new hardware settings to take effect. When the computer restarts, click on **Start**, go to **Program**s, go to **Chromatography** and select **EZChrom *Elite***.

*Figure 54 EZChrom Elite Client/Server Screen*

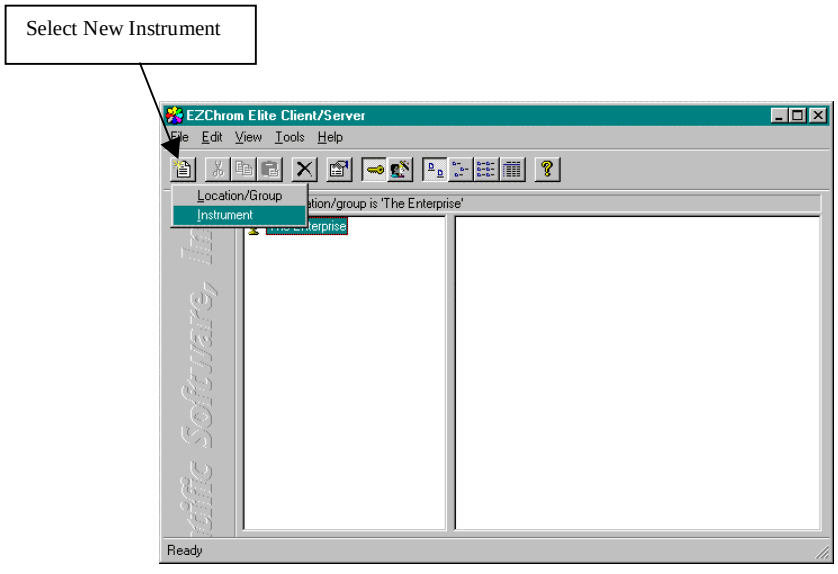


*Figure 55 EZChrom Elite Client/Server Screen*

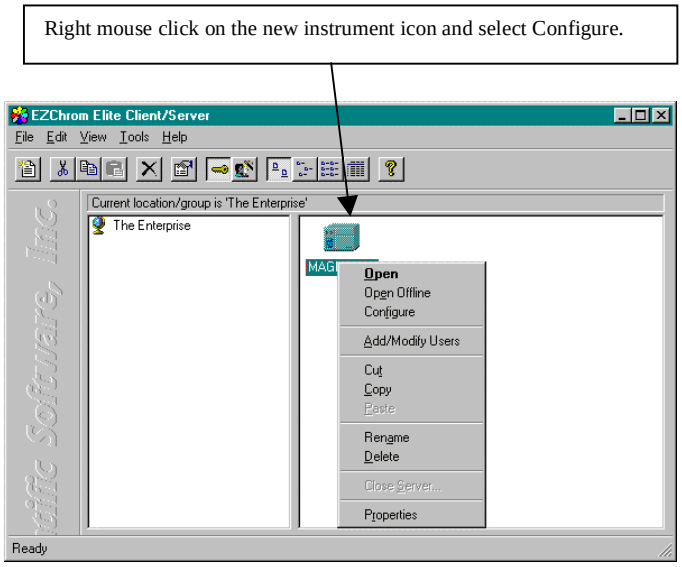



*Figure 56 Instrument Configuration Screen*

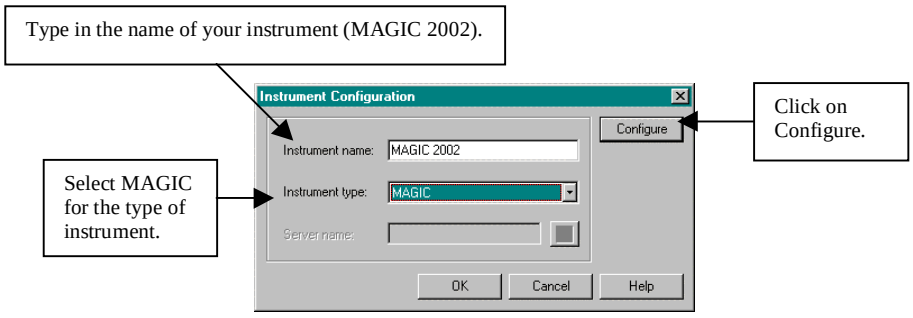
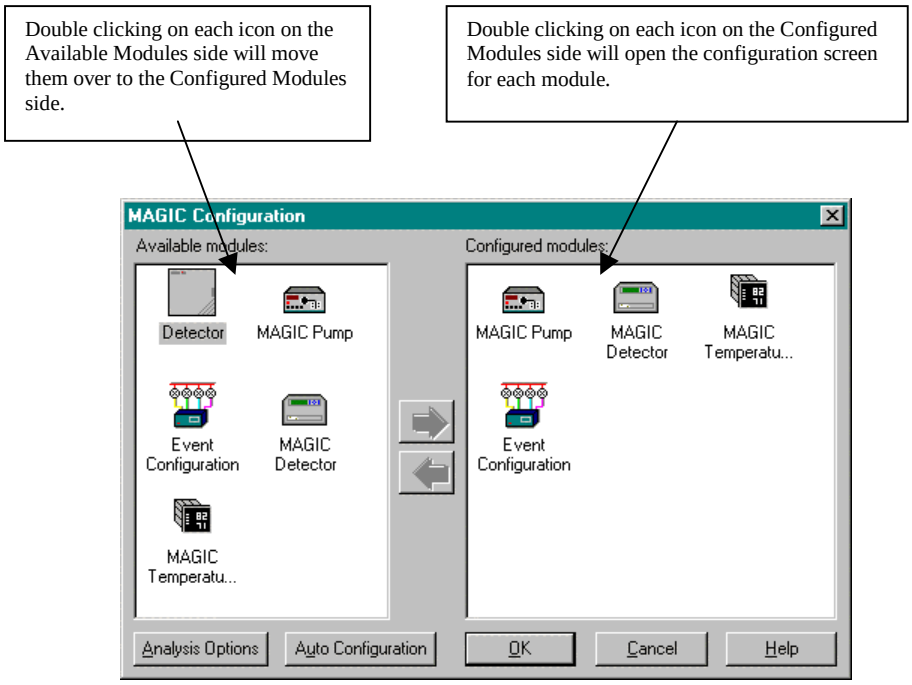
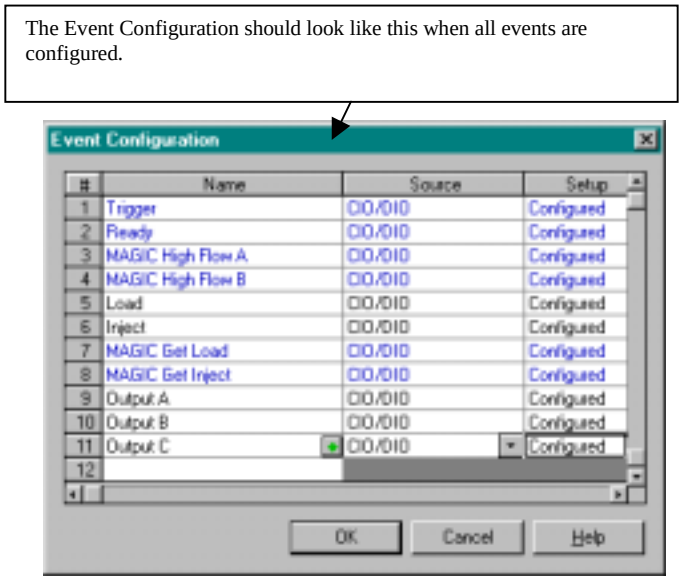


*Figure 57 MAGIC Configuration Screen*




Move the four Modules from the Available modules side to the Configured modules side as shown in Figure 57. Configure the Event Configuration first (*Refer to Figure 58 and Table 4, pages 66-67)*, and then continue on until all the modules have been properly configured as shown on the following pages (*Refer to Figures 59-61, pages 67-68)* .

*Figure 58 Event Configuration Screen*



*The following table lists the Events by Name, the Source and the Setup for each event.*

*Table 4 Control Only Event Configuration*

| Name | Source | Setup | | |
|---|---|---|---|---|
| | | Board | Input | Triggered State |
| Trigger | CIO/DIO | 0 | B0 | Closed |
| Ready | CIO/DIO | 0 | A4 | Closed |
| MAGIC High Flow A | CIO/DIO | 0 | A0 | Closed |
| MAGIC High Flow B | CIO/DIO | 0 | A1 | Closed |
| Load (must be typed in) | CIO/DIO | 0 | A2 | |
| Inject (must be typed in) | CIO/DIO | 0 | A3 | |
| MAGIC Get Load | CIO/DIO | 0 | B1 | Closed |
| MAGIC Get Inject | CIO/DIO | 0 | B2 | Closed |
| Output A (must be typed in) | CIO/DIO | 0 | A5 | |
| Output B (must be typed in) | CIO/DIO | 0 | A6 | |
| Output C (must be typed in) | CIO/DIO | 0 | A7 | |

*Figure 59 MAGIC Pump Configuration Screen*

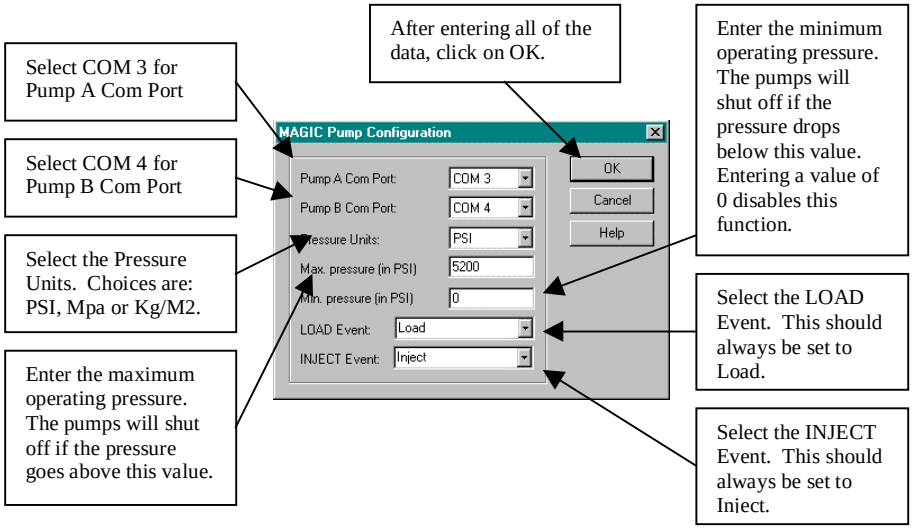


*Figure 60 MAGIC Detector Configuration Screen*

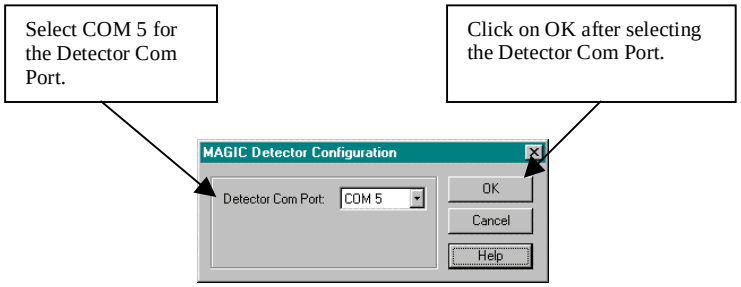
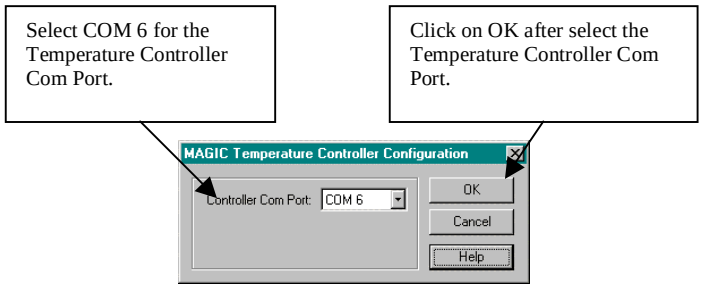



*Figure 61 MAGIC Temperature Controller Configuration Screen*




After all four modules have been configured, Click **OK** on the MAGIC Configuration screen to save these configurations and return to the MAGIC EZChrom Elite Client/Server screen. Double-click on the MAGIC 2002™ Icon to open the MAGIC software.

The contents of this operator's manual were compiled with the intent to provide the instrument user with all of the information necessary to operate the MAGIC 2002™ HPLC and the associated peripheral devices and accessories available from Michrom BioResources, Inc. Further clarification and/or additional information can be obtained from the Michrom BioResources, Inc. technical support staff at the following numbers.


| | |
|---|---|
| Telephone: | (530) 888-6498 |
| Fax: | (530) 888-8295 |
| e-mail: | michromser@psyber.com |


The Michrom BioResources, Inc. staff thanks you for your interest in our product line and look forward to serving you in the future.