

# **BIOLOGIC DUO§FLOW CHROMATOGRAPHY SYSTEM**

## **INSTRUCTION MANUAL**

**BIO-RAD**

## SAFETY

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**Caution/Warning**

Disconnect power to any BioLogic Duo-Flow system component before servicing. No user-serviceable parts are inside any component. Refer servicing to Bio-Rad service personnel.

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The BioLogic Duo-Flow system is certified to meet the I.E.C. 1010-1\* 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 Duo-Flow system.

This instrument is intended for laboratory use only.

The BioLogic Duo-Flow system 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.

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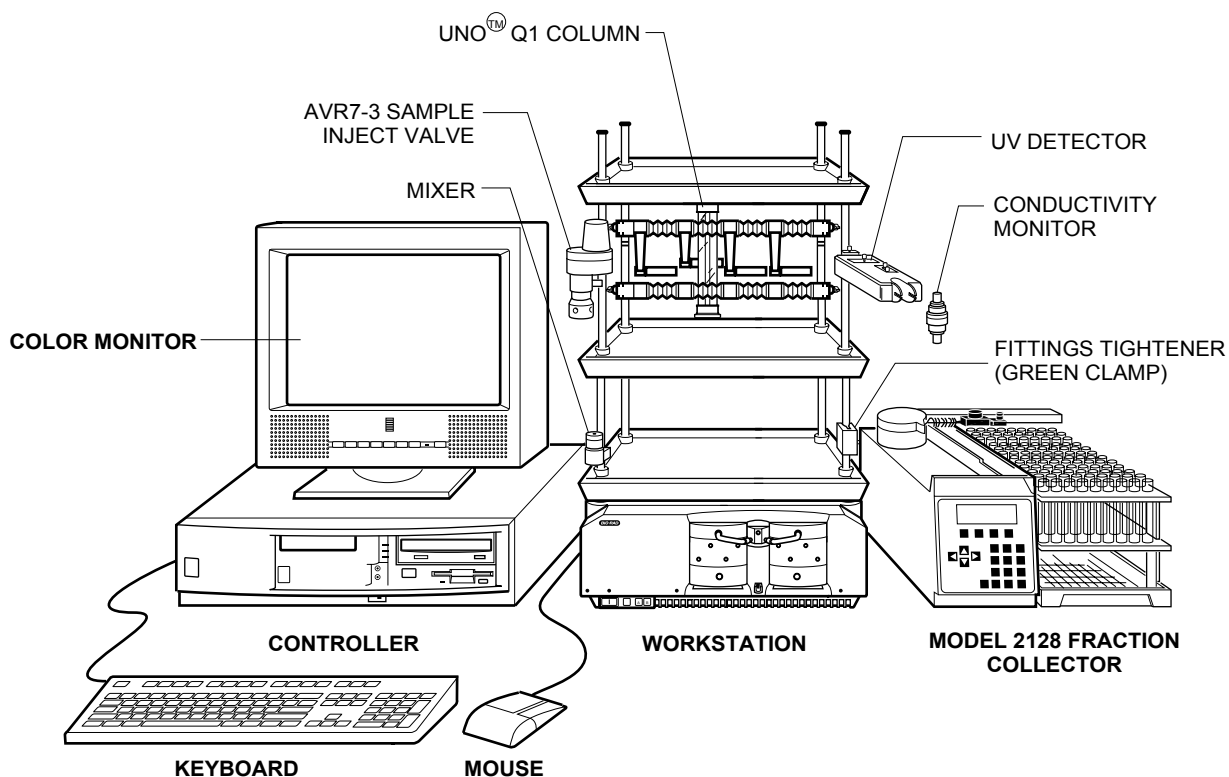
## 1.0 INTRODUCTION

### 1.1 OVERVIEW

The BioLogic Duo-Flow is a full-featured, gradient chromatography system specifically designed for the high resolution purification of proteins, peptides and other biomolecules where recovery of biological activity is of primary concern. It comes standard with an F-10 pumphead, which operates at up to 10 ml/min and 3500 psi. An optional F-40 pump upgrade is available for operation at up to 40 ml/min and 1000 psi.

The BioLogic Duo-Flow system is microprocessor controlled, with an easy-to-use graphic interface 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 1-1. BioLogic Duo-Flow System*

## 1.2 FEATURES

The BioLogic Duo-Flow system provides the following features:

- A space-saving modular and stackable design which minimizes the system footprint on the bench. The BioLogic Duo-Flow Controller, Workstation and optional peripherals are linked by a single "instrument bus" cable, making electrical connections simple and fast.
- Control software specifically designed for the chromatographer. The pre-loaded, dedicated software is based on the easy-to-use Windows® 95 platform and is designed for multiple users in the laboratory. Programming separation methods is menu-driven, easy and intuitive using either Time-based or Volume-based steps. Method storage capacity is virtually unlimited.
- On-screen help function.
- Single-point control of chart recorders, fraction collectors, signal import modules and auxiliary pumps (e.g., Bio-Rad's EP-1 Econo Pump) for sample loading.
- Binary gradient formation by both the F-10 and F-40 pumps. These pumps are interchangeable, and each consists of two dual piston, low-pulsation pump heads. The F-10 pumps operate at pressures up to 3500 psi at flow-rates between 0.01 ml/min and 10 ml/min, with optimal gradient results at flow rates between 0.1 ml/min and 10 ml/min. The F-40 pumps operate at pressures up to 1000 psi at flow-rates between 0.5 ml/min and 40 ml/min, producing optimal gradient results within these flow rates. These operating ranges permit the use of a wide range of both preparative and analytical columns.
- Dynamic mixing for accurate and reproducible gradient formation with both the F-10 and F-40 pumps.
- The use of biocompatible materials throughout the flowpath to ensure maximum recovery of biological activity.
- Standard high resolution optics unit (mercury lamp) for fixed wavelength (280 nm and 254 nm) detection and a choice of preparative or analytical flow-cells. A zinc lamp and filter combination for 214 nm detection is optional. Other filters are available from Bio-Rad.
- Standard conductivity cell to monitor gradient formation.
- Simple-to-sophisticated fraction collection schemes are standard. Collection is volume-based with Collect All, Collection Windows, Threshold and Threshold + Collection Window modes available with a range of collector types.
- Three connectors to accommodate a range of low pressure solenoid diverter and buffer select valves.
- Three connectors to accommodate a range of high pressure, automated stream select and sample injection valves. These automated valves are specially designed to eliminate pump shutdown due to high pressure spikes.
- Three-tiered System Rack to accommodate solutions, samples, and other laboratory supplies.
- Standard column clamping arrangement to organize columns of various dimensions.

### 1.3 UNPACKING

When you receive the BioLogic Duo-Flow 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 Duo-Flow system and available options.

**Table 1-1.**  
**Description of System Components**

Component	Function
<b>Controller</b>	<p>This provides the software from which the system must be set up and run. From the controller, you can set up and run methods, perform simple data analysis, and store method and run data.</p> <p>Control of system components is via the instrument bus connection. This includes control of the Workstation and its gradient pump, as well as any instruments connected to the Workstation, such as automatic valves, UV and Conductivity detectors, and peripheral instruments (such as the Model 2128 fraction collector, the Model EP-1 Econo Pump, and Signal Import Modules).</p>

**Table 1-1. (continued)**  
**Description of System Components**

Component	Function
<b>Workstation</b>	<p>This consists of the following:</p> <ul style="list-style-type: none"> <li>• Gradient Pump. There are two pump types: the F-10 and the F-40. Each consists of two bio-compatible dual-piston pump heads. Purge and Pause buttons (Purge A, Purge B, and Pause) are present on the front of the Workstation.</li> </ul> <p>The F-10 pump has a maximum delivery of 10 ml/min at a maximum pressure of 3500 psi. The F-40 pump has a maximum delivery of 40 ml/min at a maximum pressure of 1000 psi. The pump system includes a built-in pressure transducer.</p> <ul style="list-style-type: none"> <li>• Control circuitry and connectors for the UV and Conductivity Detector signals and for the Model MX-1 Mixer. Signal export connectors for UV and Conductivity data at 1 V Full Scale and for chart recorder control (pen up/down, start/stop).</li> <li>• Control circuitry and connectors for the system valves (low pressure solenoid and automated high pressure inject and select valves).</li> <li>• An Auxiliary (AUX) connector for a Model 2110 fraction collector, a generic fraction collector, an inject device, and/or a Model EP-1 Econo Pump for sample loading.</li> <li>• Output power connectors to the UV lamp and a Model 1327 Chart Recorder.</li> <li>• Two instrument bus (phone-type) connectors.</li> </ul>
<b>System Rack</b>	<p>This is the system organizer for instruments and devices used with the system, including columns and cartridges, buffer containers, sample inject and stream select valves, UV and Conductivity Detectors, and the Mixer.</p>
<b>Mixer</b>	<p>The Model MX-1 Mixer improves the quality of the gradient by mixing the output of the gradient pumps. The MX-1 Mixer comprises a Mixer Body, Standard Mixer Barrel, and Mixer Barrel Extender. The total mixing volume with the Mixer Body is 263<math>\mu</math>L, 750<math>\mu</math>L with the Standard Mixer Barrel, and 2mL with the Mixer Barrel Extender.</p>
<b>UV Detector</b>	<p>The UV Detector with a mercury lamp and filters for fixed wavelength UV detection at 280 nm and 254 nm is provided with the system. An optional zinc lamp with a 214 nm filter is available, as are filters for other wavelengths.</p> <p>The UV detector uses the following two flow cells:</p> <ul style="list-style-type: none"> <li>• An analytical "Z" cell for high resolution protein chromatography applications and low flow rates (less than 1.0 ml/min). It has a path length of 5 mm for maximum sensitivity and a volume of only 16 <math>\mu</math>L. It can be used with flow rates from 0.1 to 10 ml/min.</li> <li>• An optional cuvette cell for preparative work not requiring high sensitivity, or when working with high protein concentrations, and for flow rates greater than 10 ml/min. It has a path length of 2 mm and a volume of 30 <math>\mu</math>L.</li> </ul>
<b>Conductivity Detector</b>	<p>The Conductivity flow cell, which rests in a specially designed holder on the front of the UV Optics module, provides instantaneous, on-line readings of conductance for monitoring a salt gradient and optimization of column chromatographic conditions. It has a nominal volume of 6 <math>\mu</math>L.</p>
<b>pH Monitor</b>	<p>The pH Monitor, which is available as an option, provides real-time monitoring of buffer pH. It is designed for flow-through pH readings.</p>

**Table 1-1. (continued)**  
**Description of System Components**

Component	Function
<b>Valves</b>	<p>An AVR7-3 Inject Valve is provided with the system. Other valves available as options:</p> <ul style="list-style-type: none"> <li>• High pressure (to 3500 psi) valves: AVR7-3 Inject Valve and AVR9-8 Stream Select Valve.</li> <li>• Low pressure (to 20 psi) solenoid valves: SV5-4 Buffer Select and SVT3-2 Diverter valve.</li> </ul>
<b>Sample Loops</b>	<p>The following sample loops are available for use with the inject valves: 100 µl, 250 µl and 500 µl, 1.0 ml, 2.0 ml and 5.0 ml. In addition, the DynaLoop 25 and DynaLoop 90 Sample Loops are available for automated introduction of large sample volumes. The 100 µl, 250 µl, and 500 µL sample loops are available in a Small Volume Sample Loop Kit. The 1.0 ml, 2.0 ml, 5.0 ml sample loops are available in a Large Volume Sample Loop kit.</p>
<b>Columns</b>	<p>Bio-Rad makes a number of different column chemistries. A description of each type of column is located in Chapter 2, section 2.7, Columns and Column Fittings.</p>
<b>Auxiliary Load Pump</b>	<p>The Model EP-1 Econo Pump is available as a system option for loading large sample volumes onto low pressure columns. <b>Note:</b> To load large volumes onto high pressure columns, use Bio-Rad's DynaLoop sliding-piston sample loop.</p>
<b>Fraction Collector</b>	<p>Bio-Rad offers the following two fraction collectors as system options and supports other fraction collectors as discussed below.</p> <ul style="list-style-type: none"> <li>• The Model 2128 provides X-Y motion drop dispensing over a variety of racks. The racks accommodate a wide range of tube diameters and lengths, microtiter plates, micro-tubes, and "bottle size" fractions. The Model 2128 is suited for both analytical and preparative applications. It is controlled by the system's Controller via the instrument bus.</li> <li>• The Model 2110 uses a stationary drop-dispensing head to collect up to 80 fractions in a carousel. To divert non-peak material to waste, use the SVT3-2 Diverter valve. The Model 2110 is controlled by the system's Controller via the AUX connector on the Workstation.</li> <li>• Other fraction collectors may be used with the system as long as tube advances can be initiated by an active low pulse (&gt;100 ms). (Refer to the fraction collector's documentation for discussion of its active low pulse.) The SVT3-2 Diverter valve is required for full operation. Fraction advance marks (event marks) for a generic collector are embedded in the UV signal sent from the Workstation to a chart recorder. Generic collectors are controlled by the system's Controller via the AUX connector on the Workstation.</li> </ul> <p><b>Regardless of which fraction collector is chosen, the fraction volume and collection parameters such as Collect All, Collection Windows, Threshold, and Threshold + Collection Windows can be controlled from the BioLogic Duo-Flow Controller.</b></p>
<b>Chart Recorder</b>	<p>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 system's Controller via the Workstation. Chart speed is set at the recorder itself (the system's Controller does not control this function). Event marks including fraction advance marks are embedded in the UV signal sent from the Workstation to the chart recorder.</p> <p>The BioLogic Duo-Flow system may be used with any generic single or dual-pen chart recorder, provided the chart recorder accepts a signal between 0 and 1 V.</p>

**Table 1-1. (continued)**  
**Description of System Components**

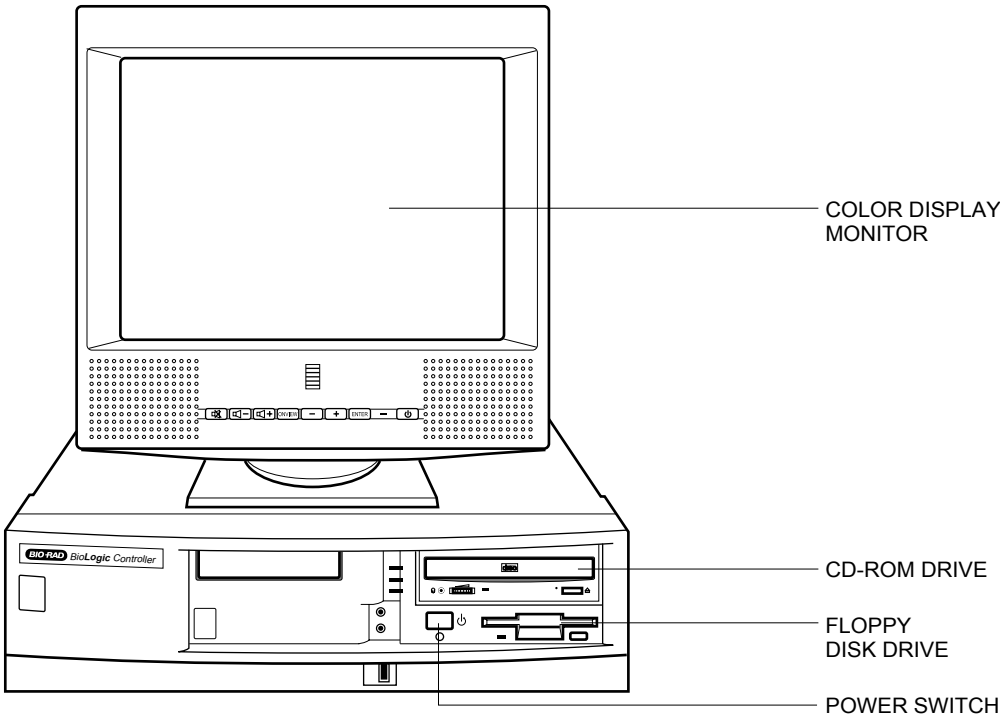
Component	Function
<b>Signal Import Module (SIM-HR)</b>	<p>Two Signal Import Modules (SIMs-HR) may be connected to the system via the instrument bus. This module allows you to connect the pH Monitor to the system. There is also a 3-pin connector which may be used to connect other detectors (such as variable wavelength, UV, fluorescence, and RI detectors) which transmit an analog signal of up to 2.5 V.</p> <p>The SIM-HR converts the detector's analog signal to a digital signal and communicates the information to the BioLogic Duo-Flow system.</p>
<b>Tubing</b>	<p>The following tubing is used with the system:</p> <ul style="list-style-type: none"> <li>• <b>Pre-pump tubing:</b> 1/8" (3.2 mm) OD, 0.062" (1.6 mm) ID PTFE tubing. The approximate volume of 1 cm of tubing is 20 µl.</li> <li>• <b>Post-pump tubing for the F-10 pump:</b> 1/16" (1.6 mm) OD, 0.020" (0.51 mm) ID orange PEEK tubing. The approximate volume of 1 cm of tubing is 2 µl.</li> <li>• <b>Post-pump tubing for the F-40 pump:</b> 1/16" (1.6 mm) OD, 0.030" (0.76 mm) ID green PEEK tubing. The approximate volume of 1 cm of tubing is 4.5 µl.</li> </ul>
<b>Fittings</b>	Flangeless 1/4-28 flat bottom fittings.
<b>Auxiliary Pump</b>	Bio-Rad's Model EP-1 Econo Pump is available as a system option. It is a two-channel bidirectional variable speed peristaltic pump for low-pressure chromatography. It offers a full range of features to facilitate ease of use as a stand-alone pump or as an accessory to the Biologic Duo-Flow system to load large volumes, especially onto low-pressure columns.

## 2.0 DESCRIPTION OF THE BIOLOGIC DUO-FLOW SYSTEM

### 2.1 CONTROLLER

The Controller provides the software from which the system must be set up and run. It is used with the Color Display Monitor, the keyboard and mouse devices, the CD-ROM drive, and the floppy disk drive which are provided with the system. **Note:** To use the sound capabilities of the CD-ROM drive, and the audio and mic connectors on the back of the Controller, refer to the documentation for the peripheral with speakers.

**Table 2-1.**  
**Controller's Front Panel Features and Color Display Monitor**

	
Feature	Description
CD ROM Drive	Use this drive to load updates of the BioLogic Duo-Flow operating software. To open the drive, press the button on the front of the drive.
Keyboard & Mouse	These two interface devices (not shown) are used to control the system. They are standard PC-compatible input devices.
Floppy Disk Drive	Use it to backup methods to - and restore methods from - floppy disk. Press the button next to the drive slot to manually eject a floppy disk from the drive.
Power Switch	Turns on/off the Controller and Monitor.

**Table 2-2.**  
**Controller's Rear Panel Connectors**

Connector	Description
	<b>Instrument bus connector:</b> The Instrument Bus allows all components in the BioLogic Duo-Flow System to communicate with the Controller. The components may be cabled together in any order via their respective Instrument Bus connectors. This is termed "daisy-chaining." The presence of a component on the Instrument Bus is recognized when it is switched on. Even when one component is switched off, another component "daisy-chained" to it can be controlled by the Controller. Note that the Controller has only one Instrument Bus connector.
	<b>Video connector:</b> Use this connector to connect the Color Monitor to the Controller.
	<b>Keyboard connector:</b> Use this connector to connect the keyboard to the Controller. It supports any standard PC compatible keyboard.
	<b>Mouse connector:</b> Use this connector to connect the mouse to the Controller. It supports any standard PC compatible mouse or trackball.
	<b>Parallel connector:</b> Use this connector when connecting any parallel printer. Laser and dot matrix printers are examples of parallel printers. Refer to Windows® 95 and/or your printer documentation for instructions on how to set up the printer for use with the BioLogic Duo-Flow System. Some printer drivers are pre-installed on the Controller.
	<b>Power Connector:</b> This is the power connector for the Controller.

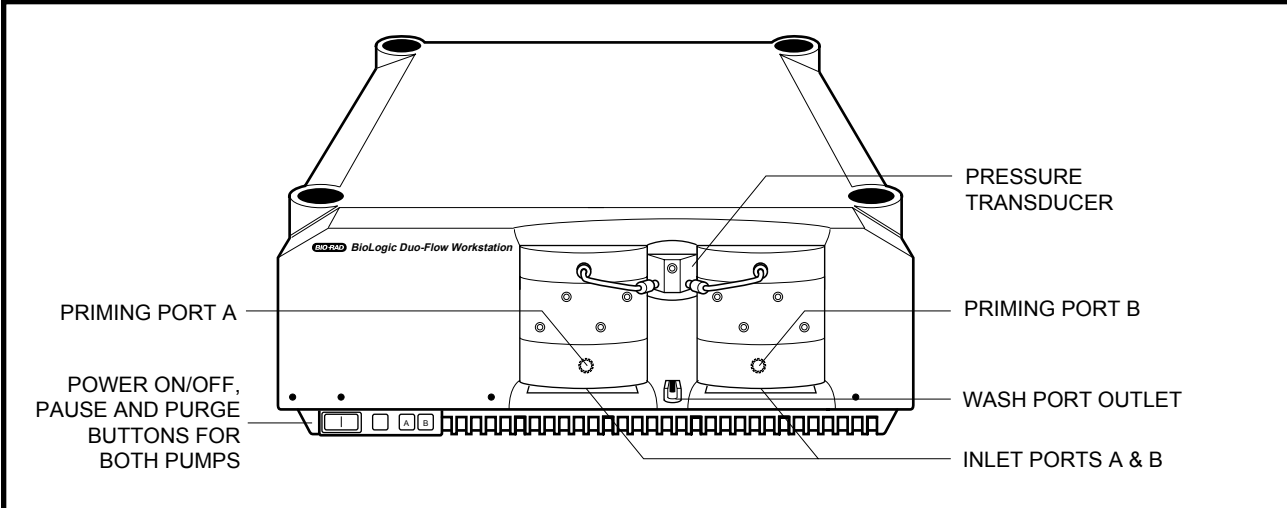


## 2.2 Workstation

The Workstation contains the following:

- Gradient Pump. There are two types of pumphead: F-10 and F-40. Each consists of two bio-compatible dual-piston pump heads. The F-10 pump supplied with the system is rated to 3,500 psi (maximum), with a flow rate range of 0.01 to 10 ml/min. The optional F-40 pump is rated to 1,000 psi (maximum), with a flow rate range of 0.5 to 40 ml/min.
- Integrated pressure transducer.
- The control circuitry for the Gradient Pump, Mixer, UV and Conductivity Detectors, and valves. Connectors for all valves and detector inputs are on the rear panel along with output connectors for UV and Conductivity signals to a chart recorder.
- An AUX combicon connector used for (a) controlling fraction advances of the Model 2110 and generic fraction collectors, (b) receiving an open/closed signal from a device such as a manual inject valve, and (c) starting and stopping the Model EP-1 Econo Pump for sample loading.
- Power supply for the Gradient Pump, the Mixer, the UV optics unit, the Conductivity cell, and the Workstation electronics.

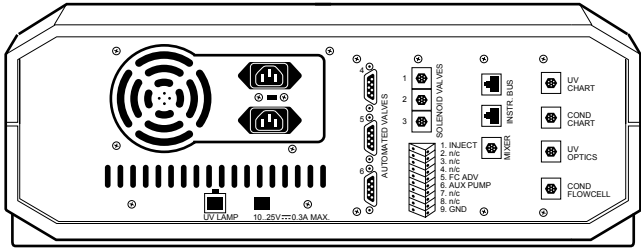
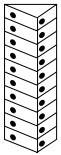







**Table 2-3.**  
**Workstation's Front Panel Controls**

	
Feature	Description
<b>Power Button</b>	Turns power on/off to the Workstation and to the components connected to it.
<b>Pause Button</b>	<p>Stops the Gradient Pump and pauses a running Method. When the Pause button is pressed, the status LEDs for pumps A and B change from green to flashing red. This button is useful in the event of last minute changes or corrections. To resume the run,</p> <ul style="list-style-type: none"> <li>• Press the button again, which restarts the pumps and the Method, or</li> <li>• Press the <b>Continue</b> button in the Run screen, or</li> <li>• Press the <b>START</b> button in the Manual screen.</li> </ul>






**Table 2-3. (continued)**  
**Workstation Front Panel Controls**

Feature	Description
<b>Purge A &amp; B Buttons</b>	<p>These buttons are used to purge the tubing lines during setup of the system.</p> <p>When the purge buttons are pressed, the pump heads run at their (default) maximum flow (F-10 purge rate is 10.0 ml/min; F-40 purge rate is 40 ml/min.) To alter the purge flow rate, select <b>Manual Setup</b> from the <b>Options</b> drop-down menu. When the buttons are pressed, the green LEDs for pumps A and B flash. To stop the flow, press the purge buttons again and note the LEDs go off. These buttons do not operate when the system is running a Method.</p> <p><b>Note:</b> Using the system software <b>Manual</b> screen, move the AVR7-3 Inject Valve to the Purge position before purging the system, so that the column is not exposed to high pressures and flow rates.</p>
<b>Alert Light</b>	<p>This LED serves two functions:</p> <ol style="list-style-type: none"> <li><b>Constant red:</b> Indicates that the pump has shut down due to a mechanical or electrical problem. This includes a shutdown due to the pressure limit being exceeded.</li> <li><b>Flashing red:</b> This occurs during a Method's run and indicates that a pre-programmed ALARM step has been reached. The system operator must respond before the method continues. Also, the Controller emits an audible tone.</li> </ol>
<b>Plumbing Connections</b>	<p><b>Ports on the front of the Workstation:</b></p> <ol style="list-style-type: none"> <li><b>Pump Head Inlet ports:</b> Buffer inlet lines attach to the ports at the bottom of each pump head. These ports accept standard 1/4-28 flat-bottom fittings. The pump head inlet tubing is 1/8" (3.2 mm) OD, 0.062" (1.6 mm) ID PTFE tubing with flat bottom fittings which are supplied in the fittings kit.</li> <li><b>Pump Head Output and Pressure Transducer Input and Output ports:</b> All plumbing following the pump head output ports uses standard 1/4-28 flat-bottom fittings and the following tubing:  <b>F-10 pumps:</b> 1/16" (1.6 mm) OD, 0.020" (0.51 mm) ID orange PEEK tubing.  <b>F-40 pumps:</b> 1/16" (1.6 mm) OD, 0.030" (0.76 mm) ID green PEEK tubing.</li> <li><b>Pump Head Priming port:</b> There is a port on each pump head which is used when priming the pump. The port accepts any size syringe with a luer fitting; a syringe is included in the Fittings Kit. This draws buffer along the inlet line and to the pump head. Twist the port counter-clockwise to open it.</li> <li><b>Pump Head Washout port:</b> The angled port at the top of each pump head is used for rinsing the piston behind the piston seals to remove crystallized salts. It accepts any syringe with a luer (not luer lock) fitting. A syringe for this purpose is included in the Fittings Kit. The rinse output is an open trough between the pump heads.</li> </ol>

**Table 2-4.**  
**Workstation's Rear Panel Connectors**

																					
Connector	Description																				
 <p>AUX 1. Inject 2. n/c 3. n/c 4. n/c 5. FC Adv 6. AUX Pump 7. n/c 8. n/c 9. Gnd</p>	<p><b>Aux connector:</b> This 9-pin connector is used to connect a variety of peripheral modules which do not communicate with the BioLogic Duo-Flow Controller over the Instrument Bus.</p> <table border="1"> <thead> <tr> <th>Pin #</th><th>Description</th></tr> </thead> <tbody> <tr> <td>1</td><td><b>Inject.</b> A contact closure between pins 1 and 9 (GND) satisfies a Hold command which has been programmed in a Method Protocol.</td></tr> <tr> <td>2</td><td><b>n/c.</b> No connection</td></tr> <tr> <td>3</td><td><b>n/c.</b> No connection</td></tr> <tr> <td>4</td><td><b>n/c.</b> No connection</td></tr> <tr> <td>5</td><td><b>FC Adv.</b> Model 2110 and generic fraction collector Advance output.</td></tr> <tr> <td>6</td><td><b>AUX Pump.</b> A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1).</td></tr> <tr> <td>7</td><td><b>n/c.</b> No connection</td></tr> <tr> <td>8</td><td><b>n/c.</b> No connection</td></tr> <tr> <td>9</td><td><b>GND.</b> Ground</td></tr> </tbody> </table>	Pin #	Description	1	<b>Inject.</b> A contact closure between pins 1 and 9 (GND) satisfies a Hold command which has been programmed in a Method Protocol.	2	<b>n/c.</b> No connection	3	<b>n/c.</b> No connection	4	<b>n/c.</b> No connection	5	<b>FC Adv.</b> Model 2110 and generic fraction collector Advance output.	6	<b>AUX Pump.</b> A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1).	7	<b>n/c.</b> No connection	8	<b>n/c.</b> No connection	9	<b>GND.</b> Ground
Pin #	Description																				
1	<b>Inject.</b> A contact closure between pins 1 and 9 (GND) satisfies a Hold command which has been programmed in a Method Protocol.																				
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7	<b>n/c.</b> No connection																				
8	<b>n/c.</b> No connection																				
9	<b>GND.</b> Ground																				
	<b>Solenoid Valves:</b> For connecting Bio-Rad's low pressure solenoid valves (SV5-4 and SVT3-2) to the system.																				
	<b>Automated Valves:</b> For connecting Bio-Rad's high pressure automated valves (AVR7-3 Inject and AVR9-8 Stream Select) to the system.																				
	<b>Cond Flowcell:</b> For connecting the Conductivity flow cell to the system.																				
	<b>UV Lamp:</b> This specialized 6-pin square connector provides electrical power to the mercury or zinc lamp in the UV Optics module's lamp housing.																				
	<b>UV Optics:</b> For connecting the UV Optics module to the system.																				
	<b>Mixer:</b> For connecting the Mixer to the system.																				
	<b>10...25V --0.3A Max:</b> Provides electrical power to the Model 1327 Chart Recorder.																				

**Table 2-4. (continued)**  
**Workstation Rear Panel Connectors**

Key	Description
	<p><b>Cond Chart:</b> For conductivity signal output to a single or dual pen chart recorder.</p> <p>An 8-pin mini-DIN to banana plug cable (<b>System Cable 4</b>) 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).</p> <p>The chart recorder should be set to 1 V full scale. If you are using the Model 1327 Chart Recorder, move all switches to the “green” settings.</p>
	<p><b>UV Chart:</b> 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 connector.</p> <p>The Bio-Rad Model 1327 dual pen recorder needs an 8-pin mini-DIN to standard DIN cable (<b>System Cable 2</b>) and a mini-DIN to banana plugs cable (<b>System Cable 4</b>).</p> <p>Generic chart recorders require an 8-pin mini-DIN to breakout cable (<b>System Cable 7</b>), available from Bio-Rad.</p> <p>When a Signal Import Module signal replaces the standard BioLogic UV Detector, use <b>System Cable 20</b> to control a Bio-Rad Model 1327 dual pen recorder.</p> <p>The chart recorder should be set to 1V.</p>
	<p><b>Power Cord Connector:</b> This grounded 3-prong connector is for input power to the Workstation and output power to any unit connected to the Workstation. The Workstation’s input power cord should be plugged into a 3-prong grounded power outlet.</p>
	<p><b>Instr Bus:</b> These RJ-45 modular phone connectors and their cables connect the Workstation to the other components in the system. The Instrument Bus handles all communications between the Controller and each of the components in the system. For example, the Instrument Bus may connect the Workstation to the Model 2128 Fraction Collector. Components can be connected in any order in the system.</p>
	<p><b>Test connector:</b> This connector is reserved for internal Bio-Rad use.</p>

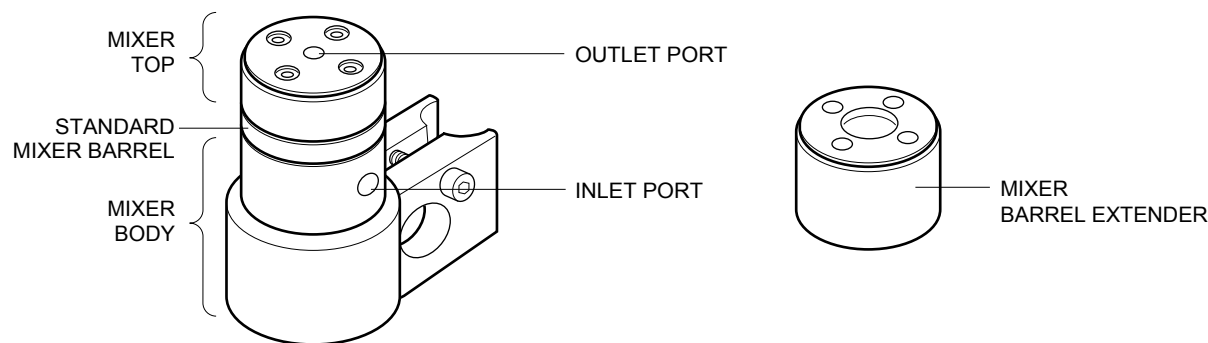
### 2.3 MX-1 MIXER

The Model MX-1 Mixer improves gradient quality by mixing the output of the BioLogic Duo-Flow gradient pumps. The Mixer cable plugs into the connector labeled “**Mixer**” on the rear of the Workstation. The Mixer has two inlet ports; seal the unused port with the plug provided.

The Mixer may be used with or without a mixer barrel: the Mixer Body, Mixer Top, and Standard Mixer Barrel are provided with the system; an optional Mixer Barrel Extender is available. The Mixer barrels fit between the mixer body and the mixer top and are used for higher flow rates. Refer to the following table to determine the appropriate mixer volume.

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

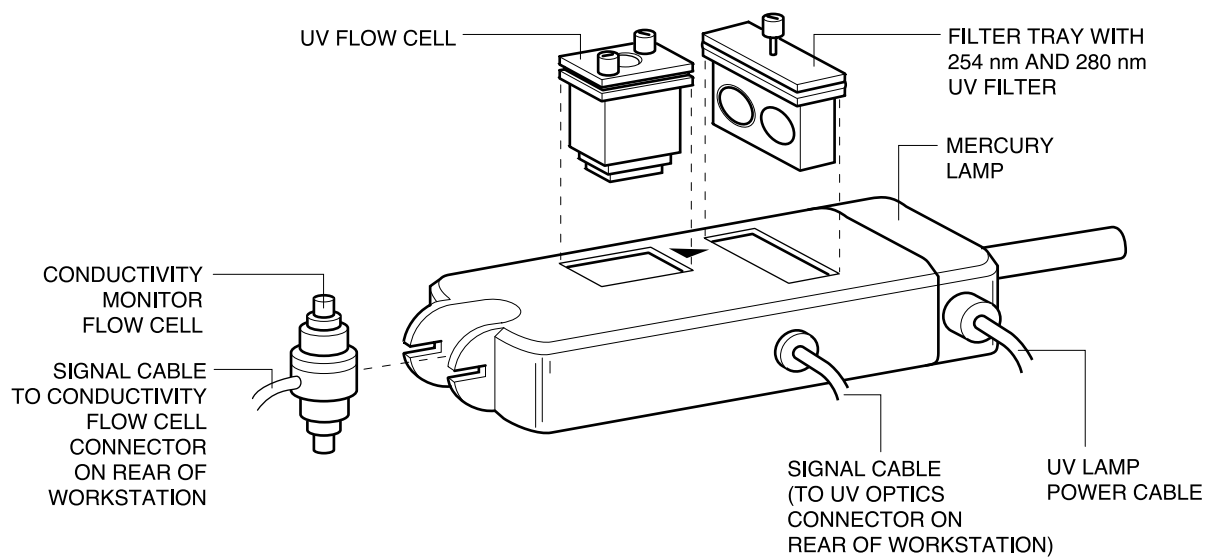
The procedure for changing the mixer capacity is provided in Chapter 3, System Setup, and in Chapter 11, Maintenance.



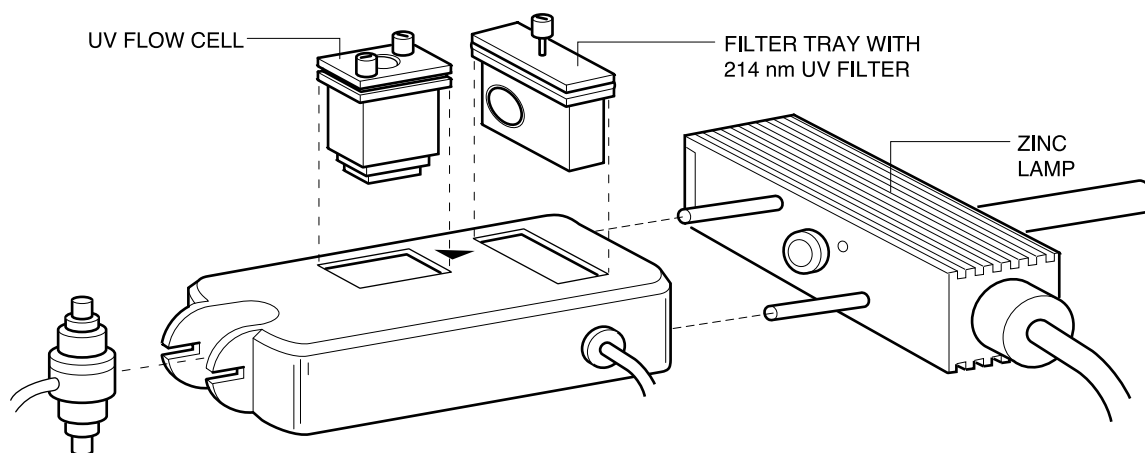
**Figure 2-1. MX-1 Mixer and Mixer Barrel Extender**

## 2.4 UV AND CONDUCTIVITY DETECTORS

The UV detector consists of the Optics bench, a filter tray, a 5 mm flow cell, and a mercury lamp. It is designed to hold the Conductivity Monitor flow cell.



**Figure 2-2. UV Optics Module, with standard Mercury Lamp, 254 & 280 nm Filters, and Conductivity Flow Cell**



**Figure 2-3. UV Optics Module, with optional Zinc Lamp, 214 nm Filter, and Conductivity Flow Cell**

### 2.4.1 UV Detector

The UV Detector is a single beam, fixed wavelength UV absorbance detector specifically designed for high resolution protein chromatography. The portability of this detector enables it to be positioned close to a column outlet, minimizing system dead volume and remixing of peaks.

The following configurations are available:

- **Mercury Lamp and 280/254 nm filters.** The mercury lamp and 280 nm and 254 nm filters are standard. The filters are both held by a single tray. To switch filters, rotate the filter holder.
- **Zinc Lamp and 214 nm filter.** The Zinc lamp and 214 nm filter are optional. The zinc lamp attaches directly to the optics module in place of the mercury lamp.
- **Mercury Lamp and other filters.** Filters for different wavelengths are also available from Bio-Rad. These include 313 nm, 365 nm, 405 nm, 436 nm, and 546 nm filters, which are supported by the mercury lamp.

The following two flow cells may be used with the system. Both use 1/4-28 flat-bottom fittings:

- **Analytical "Z" flow cell.** This flow cell is recommended for high resolution detection, for low flow rates (less than 1.0 ml/min), or where very narrow peaks are eluted (e.g., from small "polishing" columns and reverse phase columns). This flow cell has a path length of 5 mm, a volume of 16  $\mu$ l, and is rated to 750 psi at flow rates between 0.1 and 10 ml/min.  
**Note:** To reduce the risk of an entrapped air bubble causing an unstable baseline, the Z flow cell should be used with the back-pressure device. Connect the back-pressure device so that it follows the UV and Conductivity flow cells in the system plumbing.
- **"Preparative" Cuvette Flow Cell.** This flow cell is recommended for most protein purification applications which demand less sensitivity, for flow rates greater than 10 ml/min, or when working with high protein concentrations. It has a 2 mm path length, a volume of 30  $\mu$ l, and is rated to 750 psi at flow rates greater than 0.5 ml/min.

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

The UV detector sensitivity ranges from 0 to 2.0 AUFS (Absorbance Units Full Scale). The UV sensitivity range for the Chart Recorder is set in the system software in either the **Manual** or **Run** screens. A Zero Baseline button is available in the Manual and Run screens and a programmable Zero Baseline command is available as part of a Method Protocol. **Note:** Refer to the discussion of the chart recorder for setting the chart recorder range.

The UV Optics module is made up of two parts, the optics bench (which holds the flow cell and filter tray) and the lamp housing. The two sections are held together by mounting screws, two guide pins, and a gold grounding pin.

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

### 2.4.2 Conductivity Monitor

The Conductivity Monitor consists of a small portable flow cell and the control circuitry which resides in the Workstation. This monitor 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.

The Conductivity Monitor consists of the following:

- Flow cell with Inlet/Outlet ports: For connection of the tubing, use 1/4-28 flat bottom fittings. The flow cell allows flow in either direction.
- Signal Cable: For connection to the back of the Workstation. Electrical power for the Conductivity Detector is drawn off the signal cable.

The flow cell can be plumbed immediately after the UV flow cell or at any other point in the flowpath. A holder for the Conductivity Monitor is built into the UV Detector. The volume in the cell is a nominal 6  $\mu$ l.

Conductivity detector sensitivity ranges from 0 to 500 mS/cm. The Conductivity sensitivity range for the Chart Recorder is set in the system software **Manual** and **Run** screens. When setting the range, choose one that will accommodate the maximum conductivity expected during the chromatography run. Keep in mind that although the chart recorder range can be manipulated during a chromatography run, the data will be stored unattenuated, allowing for re-scaling at a later time.

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



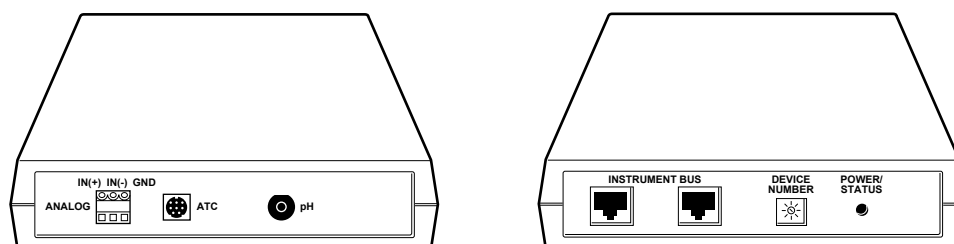
## 2.5 BIO-RAD SYSTEM OPTIONS

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

- Signal Import Module-HR (SIM-HR)
- pH Monitor
- DynaLoop Large Sample Volume Loop
- Model EP-1 Econo Pump
- Models 2110 and 2128 Fraction Collectors
- Model 1327 Chart Recorder
- BioLogic Enviro-Case
- Uninterruptable Power Supply (UPS)

### 2.5.1 Signal Import Module-HR (SIM-HR)

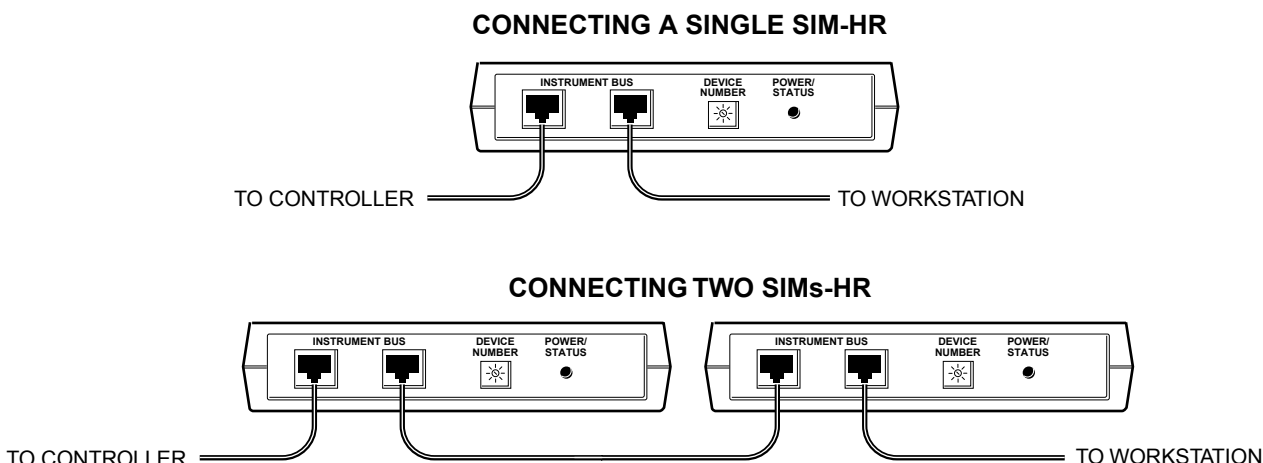
For certain chromatographic applications, it may be desirable to observe and record additional detector information. This is accomplished using the Signal Import Module-HR (SIM-HR). The SIM-HR allows for connection of both a pH monitor and another detector which outputs an analog signal of from -2.5 Volts to +2.5 Volts. Instruments that may be connected in this way could include a variable wavelength UV detector, a refractive index detector, or a fluorescence detector. The SIM-HR digitizes the analog signal and transmits it to the BioLogic Duo-Flow Controller. Two SIMs-HR may be connected to the BioLogic Duo-Flow system.



**Figure 2-4. Signal Import Module-HR (front and rear views)**

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

The instrument bus cable connects the SIM-HR to the Controller and the Workstation. (Refer to Figure 2-5.) If a second SIM-HR is to be used, one of the Instrument Bus cables is used to connect the two SIMs-HR. The SIM-HR is described in detail in its separate documentation.



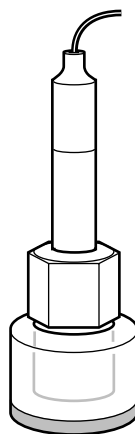
**Figure 2-5. Cable Connections to the Signal Import Module-HR**

### 2.5.2 pH Monitor

The pH Monitor provides real-time monitoring of buffer pH and is designed for flow-through pH recordings. The control circuitry for the pH Monitor resides in the Workstation. The pH Monitor consists of the following:

- Flow cell with Inlet/Outlet ports: The flow cell is plumbed immediately after any backpressure device using 1/16" (1.6 mm) OD tubing and 1/4-28 flat bottom fittings. Either port may serve as the inlet port, leaving the remaining port as the outlet port. The volume in the flow cell is a nominal 50  $\mu$ l. A holder for the pH Monitor is built into the flow cell. The flow cell should be positioned so that the flow path is angled upward to promote bubble clearance.
- pH electrode: The pH electrode is a Ag/AgCl double junction combination electrode consisting of both a pH electrode and a reference electrode built into a single body. The sealed reference design eliminates the need to add filling solutions and minimizes reference dryout. The double junction design includes a chamber filled with 3.5M KCl between the Ag/AgCl electrode and the flow stream. Consequently, this pH electrode is fully compatible with buffers (such as Tris) that would otherwise interact aberrantly with Ag/AgCl.
- Signal Cable: For connection to the SIM-HR. Electrical power is drawn off the signal cable.

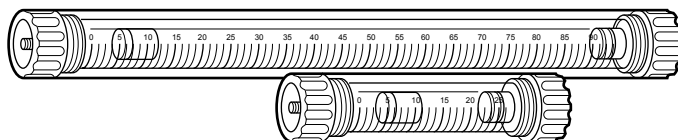
The pH electrode and flow cell are described in detail in their separate documentation.



**Figure 2-6. pH Electrode and Flow Cell**

### 2.5.3 DynaLoop Large Sample Volume Loop

The DynaLoop is a sliding-piston (dynamic) loop which allows injection of a large sample volume or repeat injections of a single sample. The DynaLoop is available in both a 25 ml and 90 ml size. It is plumbed identically to other sample loops, in ports 3 and 6 of the AVR7-3 inject valve. For instructions on how to use the high pressure inject valve with the DynaLoop, refer to Section 4, Advanced System Applications. The DynaLoop is described in detail in its separate documentation.

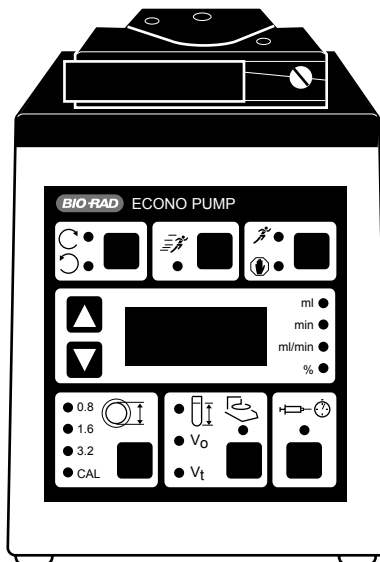


**Figure 2-7. DynaLoop**

### 2.5.4 Model EP-1 Econo Pump

The Model EP-1 Econo Pump is a two-channel, bi-directional, variable speed peristaltic pump for low-pressure chromatography. It offers a full range of features to facilitate ease of use as a stand-alone pump or as an accessory to the BioLogic Duo-Flow System to load large volumes, especially onto low pressure columns. Refer to Chapter 8, Sample Loading, for instructions on using the EP-1 pump with the BioLogic Duo-Flow System.

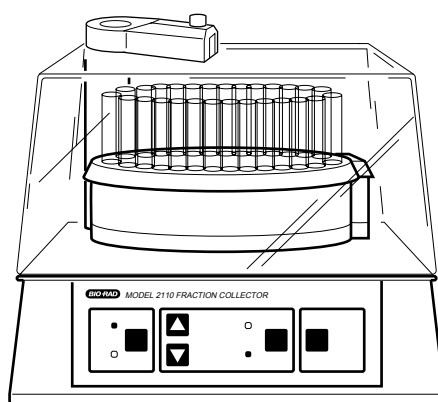
This instrument is described in detail in its separate documentation.



**Figure 2-8. Model EP-1 Econo Pump**

### 2.5.5 Model 2110 Fraction Collector

The Model 2110 uses a stationary, drop-dispensing head and 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 2-9. Model 2110 Fraction Collector with optional Dust Cover**

When controlled by the BioLogic Duo-Flow system, fraction collection is specified by volume. The fraction collection scheme is programmed in the **Protocol** screen of the software where a Delay Volume feature is standard. **Note:** Collection by time or drop count is not available with the BioLogic Duo-Flow system.

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

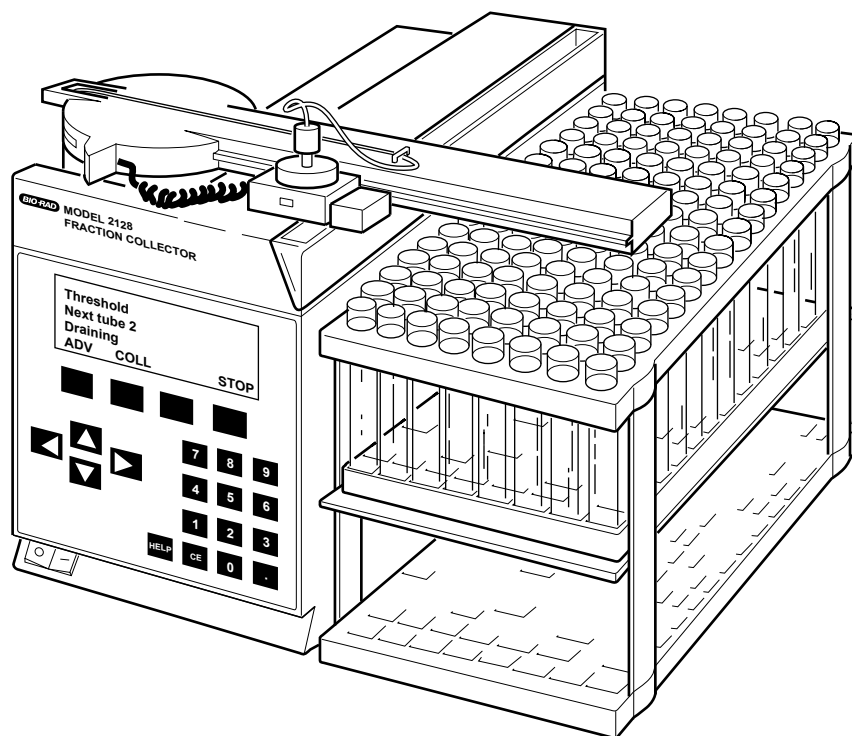
The Model 2110 is connected to the Workstation via the Workstation **AUX** connector (black wire to pin 5 and white wire to pin 9). **System Cable 5** is included for this purpose.

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

This instrument is described in detail in its separate documentation.

### 2.5.6 Model 2128 Fraction Collector

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



**Figure 2-10. Model 2128 Fraction Collector**

The following racks are available.

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

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

The Model 2128 is plumbed to the BioLogic Duo-Flow system using 1/16" (1.6 mm) OD inlet tubing and either of the following fittings:

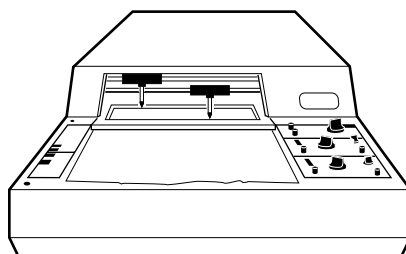
- To connect directly to the dispenser head, use 1/4-28 flat bottom fittings.
- To connect to the optional on-arm diverter valve, use the 10-32 nut and ferrule fittings supplied with the valve.

This instrument is described in detail in its separate documentation.

### 2.5.7 Model 1327 Chart Recorder

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

**Note:** The chart speed is set at the recorder. The BioLogic Duo-Flow does not control this function.



**Figure 2-11. Model 1327 Chart Recorder**

Event marks are recorded on the Chart Recorder for the following:

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

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

**2.5.8 BioLogic Enviro-Case**

The Enviro-Case is an enclosure for the BioLogic Duo-Flow's Controller and color display monitor which allows the system to be set up in a condensing, high humidity environment. It shields the moisture-sensitive components of the system from water contamination. For questions about the BioLogic Enviro-Case, consult your local Bio-Rad representative.

**2.5.9 Uninterruptible Power Supply (UPS)**

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

## 2.6 NON-BIO-RAD SYSTEM OPTIONS

Non-Bio-Rad fraction collectors and chart recorders may be used with the BioLogic Duo-Flow system, as discussed below:

### 2.6.1 Generic Fraction Collectors

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

With an optional SVT3-2 diverter valve configured as a Fraction Collector Diverter Valve in the Setup screen, the BioLogic Duo-Flow's Controller will control all collection parameters including advanced features such as collection by Threshold and/or Collection Windows. If the optional valve is not used, then only Collect All is available.

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

### 2.6.2 Generic Chart Recorders

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

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

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

### 2.6.3 Printers

Any Windows-compatible printer is compatible with the BioLogic Duo-Flow system. The printer must include a connecting cable and a printer driver (unless the printer driver is included in the Windows® disks supplied with the BioLogic Duo-Flow system).

To install a printer driver, exit the BioLogic Duo-Flow software. If the printer driver disk is available, follow the instructions that are included with that disk. If the printer driver disk is not available, click on the Windows® 95 **Start** button and from **Settings**, select **Control Panel**.

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



## 2.7 COLUMNS AND COLUMN FITTINGS

Bio-Rad offers a variety of column chemistries and formats. The following pages provide a summary of the different column types and column fittings for use with the BioLogic Duo-Flow system. Details on each of these columns is provided in Appendix E, Column Information. Bio-Rad columns for the BioLogic Duo-Flow use the following matrices:

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

### 2.7.1 Anion Exchange: Q Strong Anion Exchange

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

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

### 2.7.2 Cation Exchange: S Strong Cation Exchange

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

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

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

### 2.7.3 Anion Exchange: DEAE Weak Anion Exchange

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

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

### 2.7.4 Cation Exchange: Carboxy Methyl (CM) Weak Cation Exchange

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

- **Econo-Pac CM Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports.
- **Macro-Prep® High CM Support:** This is a weak cation exchanger containing carboxylate functional groups with a 50 µm particle size. It is ideal for purification of basic and neutral proteins and peptides. Refer to bulletins 1840 (A-300) and 1946.

### 2.7.5 Ceramic Hydroxyapatite (CHT)

The CHT chemistry is available in the following formats:

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

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

## 2.7.6 Size Exclusion Chromatography (SEC)

The SEC supports are available in the following formats:

- **Macro-Prep® SE Support:** Macro-Prep SE 100/40 and SEC 1000/40 gels are used for applications ranging from the separation of protein aggregates (dimers, trimers, etc.) from purified antibody solutions to the separation of complex mixtures of bio-molecules directly from cell culture feedstreams. SEC is commonly used as a final polishing step in the preparation of many bio-pharmaceuticals and has numerous applications as a quality control tool for assessing product purity and concentrations. These gels are made from biocompatible 40 µm agarose beads. Refer to bulletin 2190.
- **HPLC SEC Columns:** Bio-Sil and Bio-Select 5 µm silica-based columns separate compounds by the mechanism of size exclusion. The technique is based on diffusion in and around highly porous spherical silica beads. The columns are recommended for the separation of peptides, proteins, and nucleic acids, for desalting or buffer exchange, and for molecular weight or molecular constant determination. Columns are available in both stainless steel (Bio-Sil columns) and biocompatible PEEK plastic hardware (Bio-Select columns). Each column is shipped with a free vial of Bio-Rad's protein standards. Refer to bulletins 1737 and 1946.
- **Econo-Pac P6 Low Pressure Chromatography Cartridges:** This 5 ml cartridge is used for desalting and buffer exchange. Refer to bulletin 1946.

## 2.7.7 High Pressure Reversed Phase Columns

The following type of column is available:

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

## 2.7.8 Hydrophobic Interaction Chromatography (HIC)

The HIC chemistry is available in the following formats:

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

### 2.7.9 Affinity Chromatography

Affinity chromatography is available in the following formats:

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

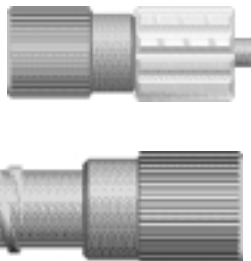
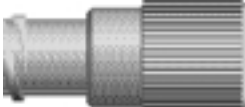
### 2.7.10 Empty Columns

The following columns are available:




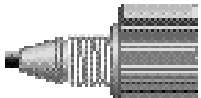
- **Bio-Scale MT empty columns:** These columns allow for easy packing of a chromatographic media, bed height adjustment, sample application, and equilibration. The availability of four column sizes (2, 5, 10, and 20 ml) allows easy scale-up of separation and purification protocols. Request bulletin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

## 2.7.11 Column Fittings

**Table 2-5.**  
**Columns and Column Fittings**

Column Type	Description	Fittings Required
<b>Econo-Pac® Cartridge</b>	The Econo-Pac cartridge has one male and one female luer-lock fitting. To connect it to the BioLogic System use 1/4-28 to male and 1/4-28 to female luer adapters, provided in the kit shown to the right. One set is included in the BioLogic Duo-Flow System Fittings Kit.	<p><b>Catalog # 732-0113</b> Econo-Pac® Cartridge to BioLogic System Fittings Kit</p> 
<b>Econo-Column®/ Low Pressure Columns and flow adapters</b>	<p>Econo-Columns have two male luer-lock fittings. To connect to the system, use female luer to 1/4-28 adapters.</p> <p>If a flow adapter with flexible tubing is to be used, attach the barbed-to-male luer fitting (included in this kit), then connect the female luer to 1/4-28 adapter.</p> <p>If the column adapter uses PTFE tubing, attach the small length of 1/16" (1.6 mm) ID Tygon® tubing and insert the barbed male luer fitting into open end. Five cm of Tygon tubing is included in this kit. Connect as above.</p>	<p><b>Catalog # 750-0565</b> Econo-Column® to BioLogic System Fittings Kit</p>  <p>This kit includes all the parts needed to connect Econo-Columns and flow adapters to the BioLogic Duo-Flow system.</p>

**Table 2-5. (continued)**  
**Columns and Column Fittings**

Column Type	Description	Fittings Required
<b>FPLC® Column</b>	Pharmacia's FPLC columns use M-6 fittings. To connect to the system, use 1/4-28 to M6 adapter union. Two of these are supplied with the BioLogic Fittings Kit.	<b>Catalog # 750-0561</b> Union, 1/4-28 to M6 
<b>HPLC Column, or Pharmacia's RESOURCE™ Column</b>	These columns accept 10/32 nuts. To connect this type of column to the system, use two 1/4-28 to 10-32 adapters.	<b>Catalog # 750-0564</b> HPLC Column to BioLogic System Adapters 
<b>UNO™ Column to FPLC system</b>	This kit includes two nuts and four ferrules to connect an UNO column to an FPLC system.	<b>Catalog #750-0567</b> M6 Fittings Kit 
<b>UNO™ Column to HPLC system</b>	This kit includes two nuts and four ferrules to connect an UNO column to an HPLC system.	<b>Catalog #750-0568</b> 10-32 Fittings Kit 

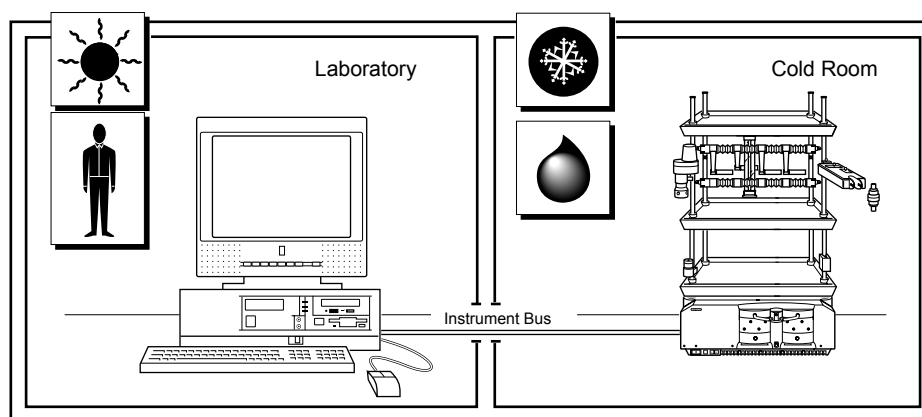


### 3.0 SYSTEM SETUP

The BioLogic Duo-Flow system is modular, so that you can configure your system in an arrangement that is most comfortable to you and best meets your application requirements. When planning a configuration, 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 Duo-Flow system can be used with Bio-Rad as well as non-Bio-Rad components.

The BioLogic Duo-Flow system can be set up wherever there is available bench space and sufficient power outlets, including in 4° cold rooms or cold boxes. For cold box or cold room use where space is limited, the compact design of the Workstation and rack and use of the single instrument bus cable to the Controller allows the Controller to be placed in a more convenient location outside the cold box (see Figure 3-1 below).

If the BioLogic Duo-Flow Controller and its Color Monitor **must** be used in a condensing environment, they should be placed in the optional BioLogic Enviro-Case protective environmental enclosure.

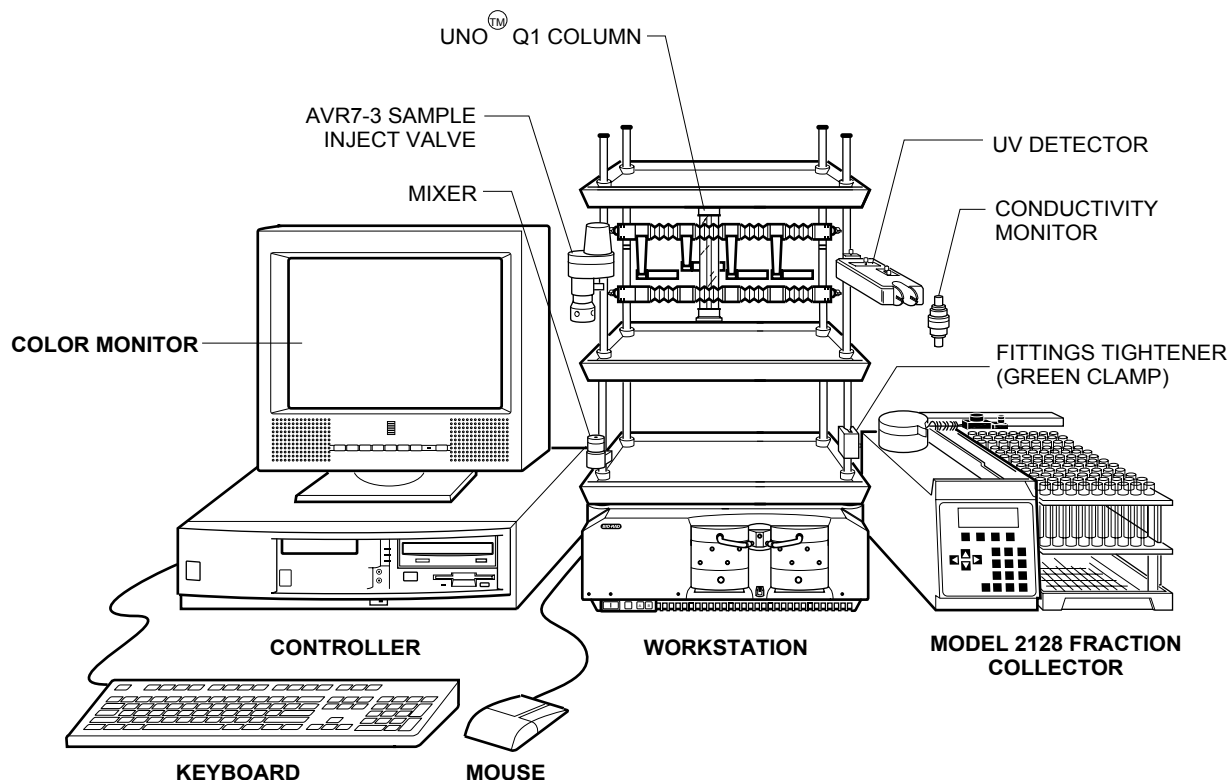


**Figure 3-1. BioLogic Duo-Flow System as it might be used with a Condensing Cold Room**

The core BioLogic Duo-Flow system includes:

- BioLogic Duo-Flow Controller and Color Monitor, keyboard, and mouse
- BioLogic Duo-Flow Workstation with F-10 pumps
- 3-Tray System Rack
- UV and Conductivity Detectors and flow cells
- Mixer module
- AVR7-3 Inject valve
- Fittings kit
- Starter kit
- Instruction Manual








*Figure 3-2. Example of a BioLogic Duo-Flow System Configuration*

### 3.1 SETTING UP THE CORE BIOLOGIC DUO-FLOW SYSTEM

Each of the following subsections discusses how to set up the core components in a BioLogic Duo-Flow system

#### 3.1.1 BioLogic Duo-Flow Controller and Color Monitor Cable Connections

1. Place the Controller on the bench with the color monitor on top, and turn both units so that the back side is facing you.
2. Connect the monitor's cable to the Controller connector marked .
3. Connect the keyboard's cable to the connector marked .
4. Connect the mouse cable to the connector marked .
5. Connect the bus cable (**System Cable 17, 18, 19, or 21**) to the connector marked "Inst Bus."
6. Connect the power cables to the Controller and monitor. **Do not turn on these devices yet.** Plug the monitor's cable into the available outlet on the Controller.

#### 3.1.2 BioLogic Duo-Flow Workstation Cable Connections

**Note:** This procedure assumes that you will be using the pump head which is installed on the Workstation. If you will be using the optional F-40 pumps, refer to the documentation provided with the F-40 Pump Upgrade Kit.

1. Place the Workstation next to the Controller with the back side facing you.

2. Note the two connectors marked “Instr Bus.” No connections are required at this time, although it is useful to note that these connectors are identical: either may be used when connecting instrument bus cables from the Controller and a Model 2128 fraction collector and/or a Signal Import Module (SIM-HR), as discussed later.
3. Confirm the voltage setting for the Workstation’s power supply. A red switch on the back of the Workstation allows you to switch between 110 V AC and 240 V AC.
4. Connect the power cable to the Workstation. **Do not turn on this device yet.**

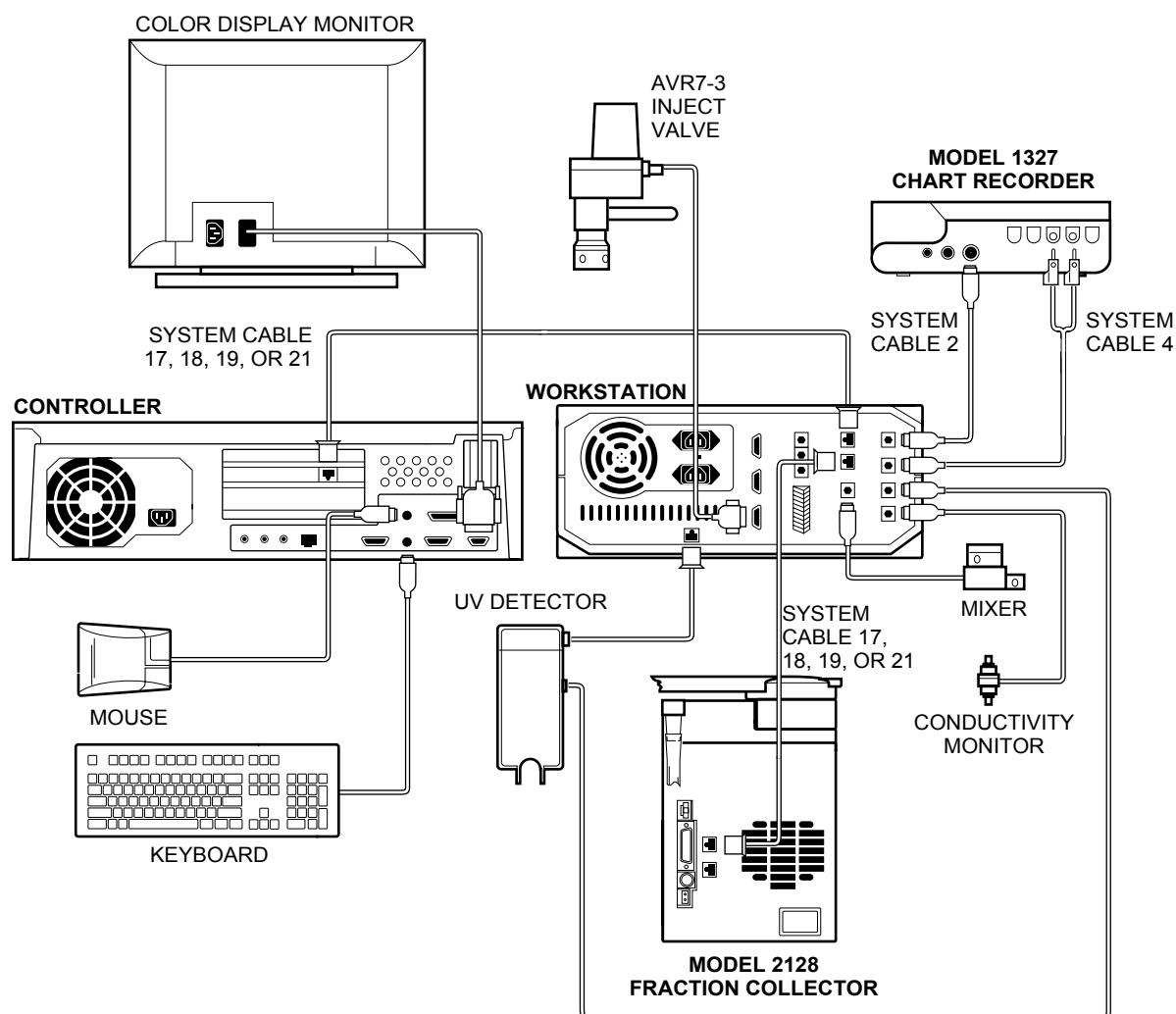
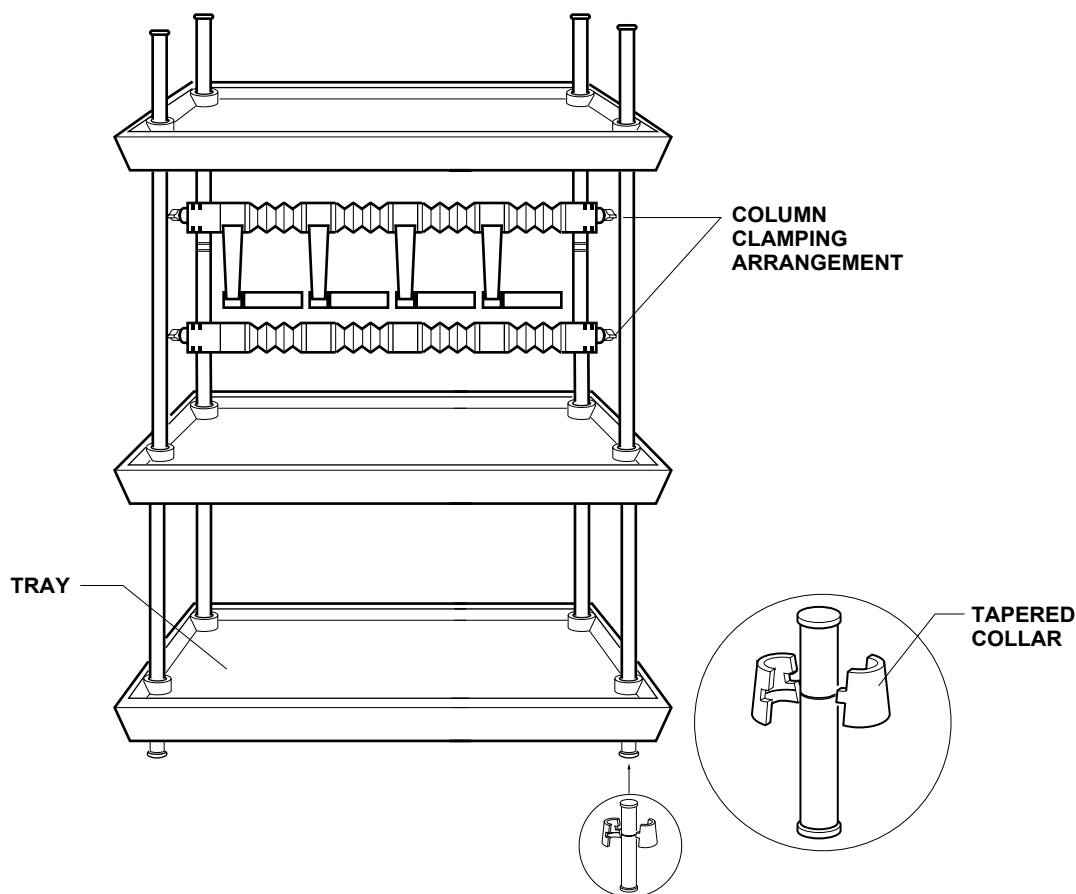


Figure 3-3. System Cable Connections

### 3.1.3 System Rack Setup

Assemble the Rack before placing it on the Workstation, as described below. Keep in mind that the illustration is only an example. Alternative rack arrangements include mounting the column clamping arrangement to the side of the rack to hold tall columns or setting up the rack to use only one or two trays.

**Note:** At any point during rack assembly, you may want to mount devices which slide onto the rack bars. Examples of rack-mount devices are discussed in subsequent steps and include the Mixer, the green metal Fittings Tightener, the UV detector, and any valves.



**Figure 3-4. Rack Assembly**

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

2. Insert the rods into the holes at each corner of a tray. Insert the rods from *underneath* the tray such that they produce a firm fit in the holes.
3. For a 2 or 3-tray rack, attach sleeves to the middle of all four long vertical bars. Remember that the sleeves should be oriented so that the wide part of the taper is nearest the rod's bottom end.
4. For a 2 or 3-tray rack, place the second tray on top of the four sleeves and press firmly to seat the tray. Repeat these instructions to add the upper tray.
5. Mount the 2-piece column clamping arrangement across the two long rods using the attached thumbscrews.
6. Two horizontal bars with rod clamps and an Allen wrench are provided with the system. These are used to hold devices, such as valves. Position them between any of the upright bars.
7. Remove the four green caps covering the holes at the four corners on top of the Workstation. Place the rack into the four corner holes.

### 3.1.4 MX-1 Mixer

The Mixer is shipped pre-assembled with the Standard Mixer Barrel. The Standard Mixer Barrel may be removed or replaced to provide the mixer capacity appropriate to the flow rate. The Standard Mixer Barrel is used for higher flow rates. Refer to the following table when selecting a mixer volume.

Flow Rate	Barrel Extension	Mixer Capacity	Assembly Screws
less than 1ml/min	none	263 $\mu$ l	10-32 x 5/8" (1.6 cm)
1 to 10 ml/min	Standard Mixer Barrel	750 $\mu$ l	10-32 x 7/8" (2.2 cm)

#### To attach the Mixer to the rack:

1. Confirm that the Mixer capacity volume is appropriate for the flow rate. If it is not, refer to the procedure below for changing the Mixer capacity.
2. Attach the Mixer to a vertical bar using its rod clamp. The Mixer should be positioned between the pump and the AVR7-3 Inject valve. Attach the Mixer to the rack so that the Mixer outlet port faces upward.
3. Connect the Mixer signal cable (mini-DIN connector) to the connector marked "**Mixer**" on the rear of the Workstation. Refer to Figure 3-3.
4. Plug the unused inlet port using the plug provided for this purpose.

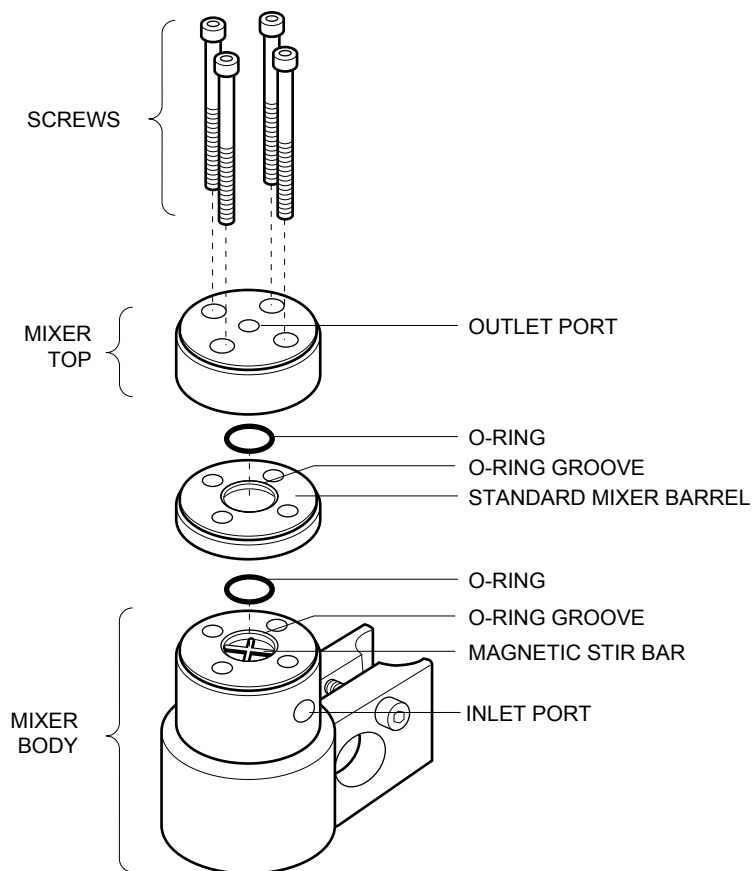
#### To change the Mixer capacity:

Be sure to follow these directions carefully. If the Mixer is assembled incorrectly, or if an O-ring is not correctly placed in the O-ring groove, then the Mixer will leak.

**Note:** If the system has been used, make sure you flush any hazardous material from the system, the pumps are not running, and any residual pressure has been bled from the system. Drain fluid from the Mixer and disconnect the Mixer plumbing and cables.

1. If you will be changing the mixer capacity, use the information in the table above to select the appropriate screws.
2. Use the hex key provided to remove the four screws from the top of the Mixer (see Figure 3-5).
3. Remove the Mixer Top and turn it upside down to remove the O-ring. If the O-ring does not easily dislodge, then use your fingers to remove it.

4. Refer to Figure 3-5 for re-assembling the Mixer. Make sure that the magnetic stir bar lies flat. If the Standard Mixer Barrel is to be used, make sure that the O-ring groove faces up.
5. Place an O-ring in each O-ring groove. If you are using only the Mixer Body (263 $\mu$ L), then only one O-ring is required. If the Standard Mixer Barrel is used, then two O-rings are required.



**Figure 3-5. MX-1 Mixer**

### 3.1.5 UV and Conductivity Detectors

Two flow cells are available for use with the UV Detector. Select the flow cell appropriate for the flow rate.

- An analytical “Z” cell for high resolution protein chromatography applications and low flow rates (less than 1.0 ml/min). It has a path length of 5 mm for maximum sensitivity and a volume of only 16 ml. It can be used with flow rates from 0.1 to 10 ml/min.
- An optional cuvette cell for preparative work not requiring high sensitivity, or when working with high protein concentrations, and for flow rates greater than 10 ml/min. It has a path length of 2 mm and a volume of 30 ml.

The procedure for connecting the Bio-Rad UV and Conductivity Detectors is described below. To connect a non-Bio-Rad UV Detector to the system, refer to section 3.3.3, Non-Bio-Rad Detectors.

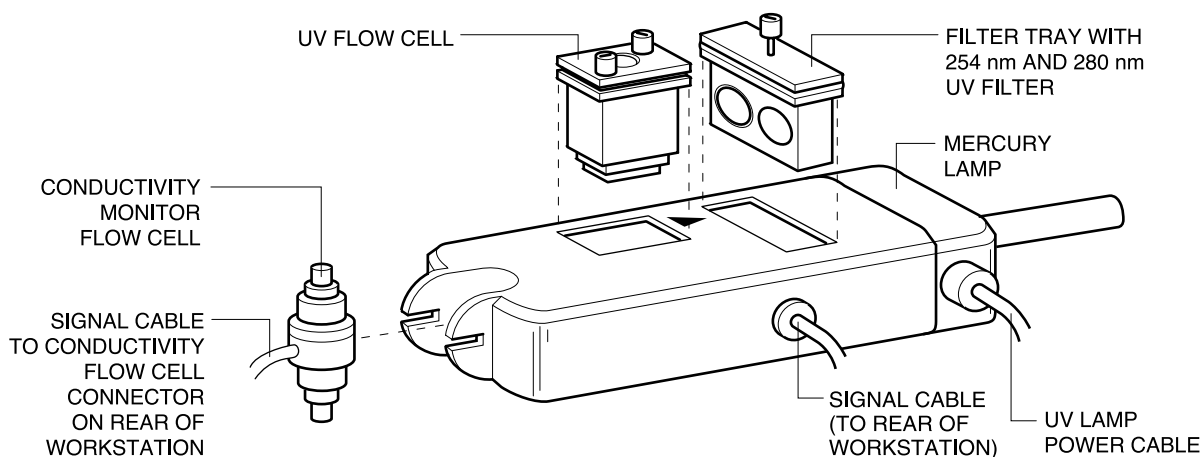
#### To attach the UV and Conductivity Detectors to the rack:

1. Using the information provided above, confirm that the UV flow cell capacity volume is appropriate for the flow rate. If it is not, refer to the procedure below for changing the UV flow cell capacity.
2. Attach the UV Detector to a vertical or horizontal bar using the rod clamps.
3. Connect the power cable (square connector) from the UV Detector lamp into the connector marked “**UV Lamp**” on the rear of the Workstation. Refer to Figure 3-3.
4. Connect the UV Detector signal cable (mini-DIN connector) to the connector marked “**UV Optics**” on the rear of the Workstation. Refer to Figure 3-3.
5. Connect the Conductivity Detector’s combined power and signal cable (mini-DIN connector) to the connector marked “**Cond. Flowcell**” on the rear of the Workstation. Refer to Figure 3-3.
6. The Conductivity Detector is designed to be held in the circular notch of the optics bench, but may be placed anywhere in the fluid path as desired by the user.

#### To change the UV flow cell or UV filter:

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

1. To remove the UV flow cell, loosen the flow cell thumbscrews and lift the flow cell out.
2. To insert a flow cell, place it into the UV detector and tighten the thumbscrews.



**Figure 3-6. Replacing the UV Detector Flow Cell**

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

### 3.1.6 Connecting Valves

The BioLogic Duo-Flow system is shipped with an AVR7-3 Inject valve. The connection of all valves is similar, as discussed below:

- To connect either the AVR7-3 Inject valve or an optional AVR9-8 Stream Select valve, mount the valve to a vertical bar on the system rack and then connect its cable to any of the connectors labeled Automated Valves 4, 5, or 6 on the rear of the Workstation.
- To connect an optional SVT3-2 Diverter valve or the optional SV5-4 Buffer Select valve, solenoid valve, mount the valve to a vertical bar on the system rack and then connect its cable to any of the connectors labeled Solenoid Valves 1, 2, or 3 on the rear of the Workstation.

## 3.2 COMPLETING THE SYSTEM SETUP

The preceding sections discussed how to set up a core system. However, the BioLogic Duo-Flow is designed to support a large number of additional instruments and devices. Section 3.3 provides the procedures for a number of different instruments and devices.

Once you have made all the appropriate connections, you can complete the system setup, as discussed below.

### 3.2.1 Arranging the System on the Bench

Once you have completed the system setup, turn the units around, connect the Instrument Bus cables, the power cords, and turn the system on. In certain laboratory environments, an uninterruptable power supply (UPS) may be required. Bio-Rad offers both 110 V and 220 V UPS configurations; consult your local Bio-Rad representative.

### 3.2.2 Connecting your Duo-Flow system to the Network (optional)

The Duo-Flow system is equipped with an Intel Ether Express Pro 100 network card. To print reports and export files over the network, install the software drivers for the network card and then set up Windows via the Network manager. Contact your site administrator to ensure proper communication and access with the existing network.

**Note:** The BioLogic software uses I/O addresses 31C and 31D. Do not use these addresses during setup.

### 3.3 CONNECTING OTHER INSTRUMENTS AND DEVICES TO THE SYSTEM

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

- Model 2110 Fraction Collector
- pH probe
- Non-Bio-Rad detectors
- Model 1327 Chart Recorder

#### 3.3.1 Model 2110 Fraction Collector

The Model 2110 Fraction Collector may be connected to the Workstation, as discussed below:

**Note:** This procedure requires the use of **System Cable 5**, a DB-9-to-bare wire cable available from Bio-Rad.

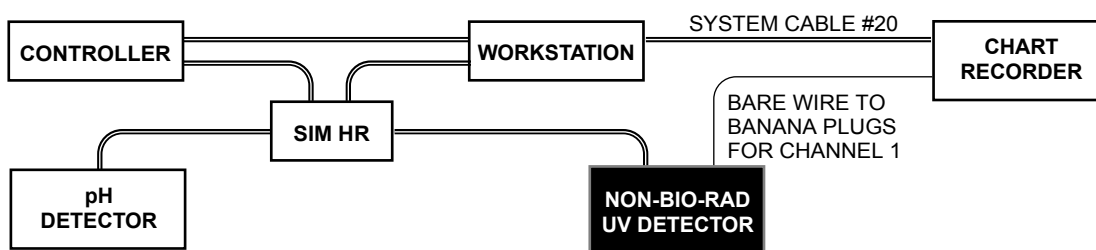
1. Connect the DB-9 connector on **System Cable 5** to the Model 2110 Fraction Collector.
2. Connect the bare wires to the **AUX** connector on the Workstation as follows: Black wire to pin 5 and white wire to pin 9.

#### 3.3.2 pH Probe

The pH probe, available as an option from Bio-Rad, may be connected to the Duo-Flow system through the use of the optional SIM-HR, also available from Bio-Rad. The SIM-HR connects to the Duo-Flow Workstation and Controller through the use of the Instrument Bus cables (**System Cables 17, 18, 19, or 21**), as shown below. The pH probe is discussed in detail in its separate documentation.

To connect the optional Bio-Rad pH probe,

1. Connect the output from the pH probe to the connector labeled **pH Connector** on the SIM-HR.
2. Connect the SIM-HR to the Duo-Flow Workstation and Controller by using the Instrument Bus cables (**System Cables 17, 18, 19, or 21**), as shown below.



**Figure 3-7. SIM-HR Connections**

#### 3.3.3 Non-Bio-Rad Detectors

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



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

#### 3.3.4 Chart Recorder (Model 1327)

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

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

## 4.0 SYSTEM PLUMBING

The devices you choose and the plumbing arrangements you make may change for each application. For this reason, this chapter begins with discussion of recommended plumbing practices and general guidelines, followed by a general procedure for plumbing the major system components. Refer to Section 4, Advanced System Applications, for valve plumbing for specific applications.

### 4.1 GENERAL GUIDELINES FOR PLUMBING THE SYSTEM

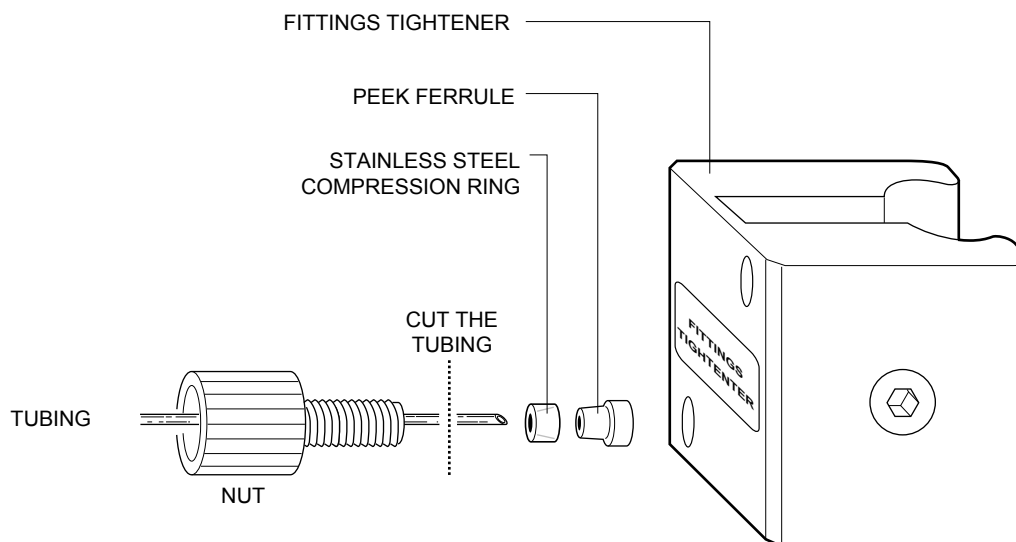
The Duo-Flow system uses three sizes of tubing. Use the following table to select the appropriate tubing.

Use	Tubing Dimensions	Tubing Material	Fittings	Vol/cm
Pre-pump	1/8" OD x 0.062" ID (3.2 mm OD x 1.6 mm ID)	clear, PTFE	Super flangeless for 1/8" (3.2 mm) OD tubing	20 µl
Post-pump with F-10 pumphead	1/16" OD x 0.020" ID (1.6 mm OD x 0.51 mm ID)	orange, PEEK	Super flangeless for 1/16" (1.6 mm) OD tubing	2 µl
Post-pump with F-40 pumphead	1/16" OD x 0.030" ID (1.6 mm OD x 0.76 mm ID)	green, PEEK	Super flangeless for 1/16" (1.6 mm) OD tubing	4.5 µl

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

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

1. Cut the tubing with the tubing cutter provided in the Fittings Kit. This will give a flat, clean cut to the tubing; this is important in making a fitting.



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

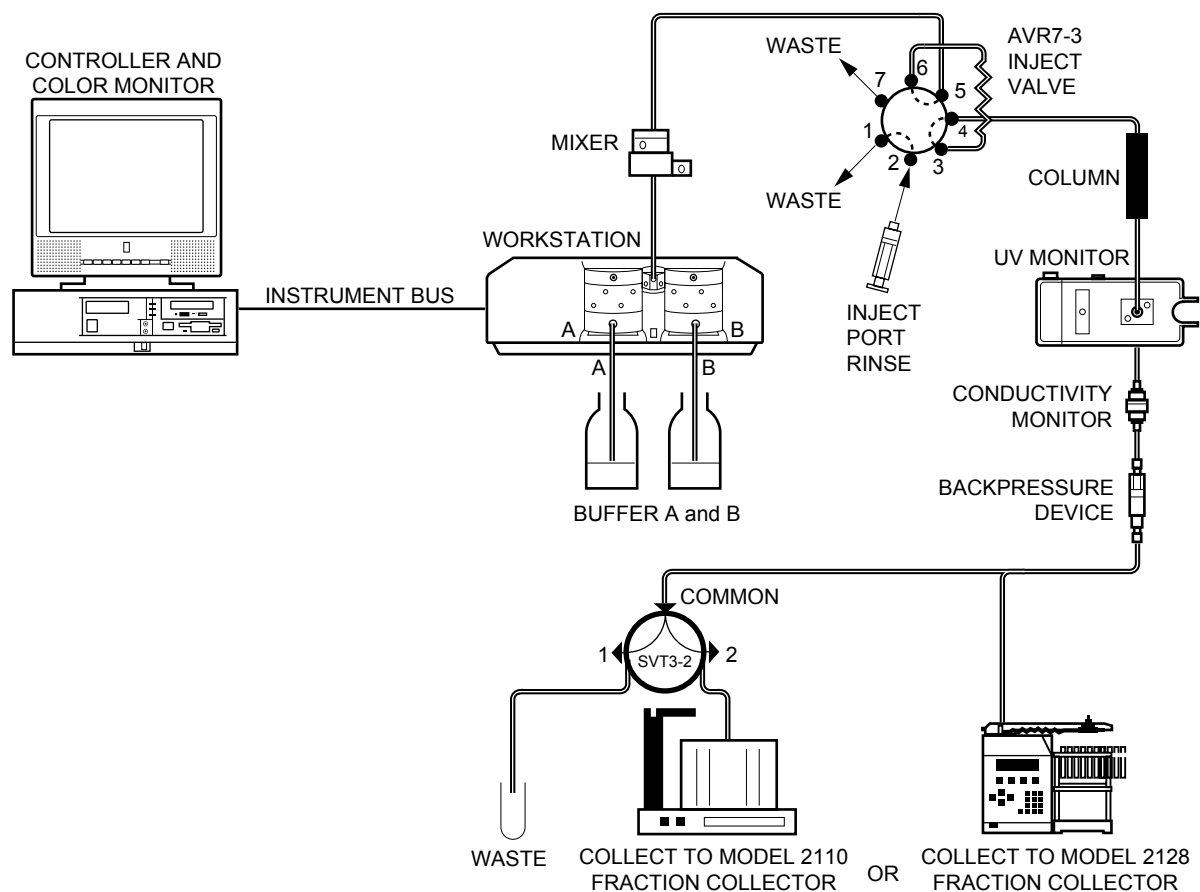
2. Slide the nut, stainless steel lock ring, and the ferrule, in that order, onto the tubing as shown above. The flattened end of the lock ring should face towards the nut with the tapered end facing the tapered end of the brown ferrule.

**Note:** The stainless steel lock ring is *not* in the fluid path, so biocompatibility is maintained.

3. Allow the tubing to extend slightly beyond the end of the ferrule.
4. Place the fitting into the green Fittings Tightener. Do not allow the tubing to slip out of the ferrule.
5. Use the Fittings Tool (supplied in the Fittings Kit) to seat the ferrule onto the tubing, but do not over-tighten.
6. Once the fitting is made, it can be inserted into the port.

## 4.2 PLUMBING THE CORE BIOLOGIC DUO-FLOW SYSTEM

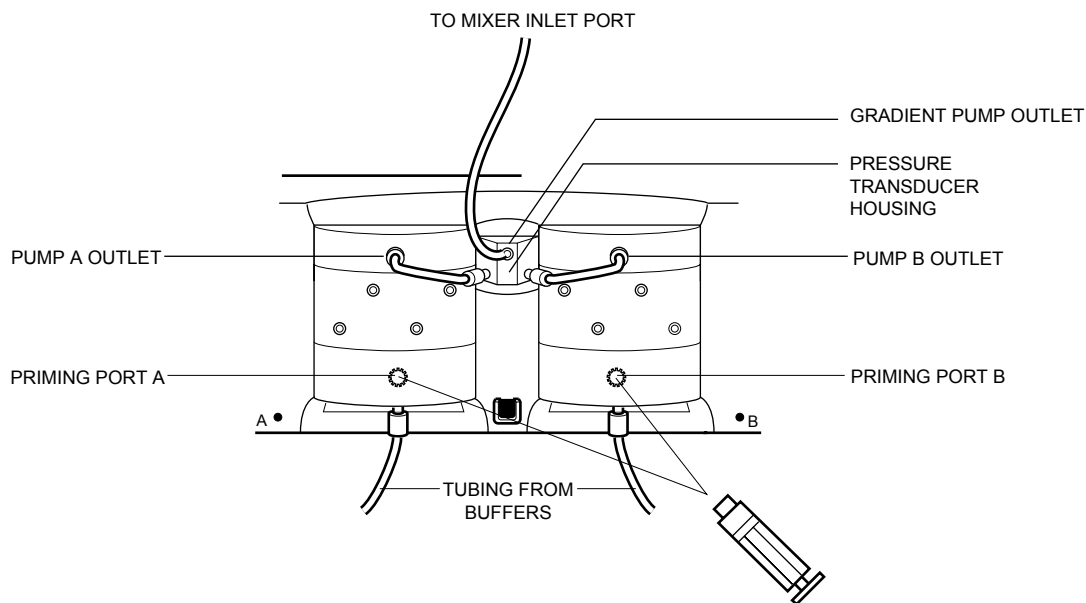
This section discusses the plumbing of the BioLogic Duo-Flow core system which was set up in the previous chapter. The plumbing for each of the optional Duo-Flow valves is discussed later in the chapter.



**Figure 4-2. System Plumbing**

**1. Plumbing the Workstation Gradient Pump Inlets to the Buffer Reservoirs (Figure 4-3).**

- Make two fittings using 1/8" (3.2 mm) OD tubing and fittings (at one end only) as described earlier. (See 4.1, General Guidelines for Plumbing the System.)
- Screw the tubing into the inlet connectors on the bottom of the Workstation Gradient Pump housing. **Ensure a firm connection but do not over-tighten.**
- Immerse the inlet lines in a container of high quality, filtered water (e.g., HPLC grade) or buffer.



**Figure 4-3. Plumbing Connections to the Gradient Pump**

**2. Plumbing from the Gradient Pump pump heads to the Transducer (Figure 4-3).**

- Make two fittings using 1/16" (1.6 mm) OD tubing and fittings, as described earlier.
- Connect the tubing to each pump head outlet port and to each transducer inlet port and secure firmly using a twisting action. **Ensure a firm connection but do not over-tighten.**

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

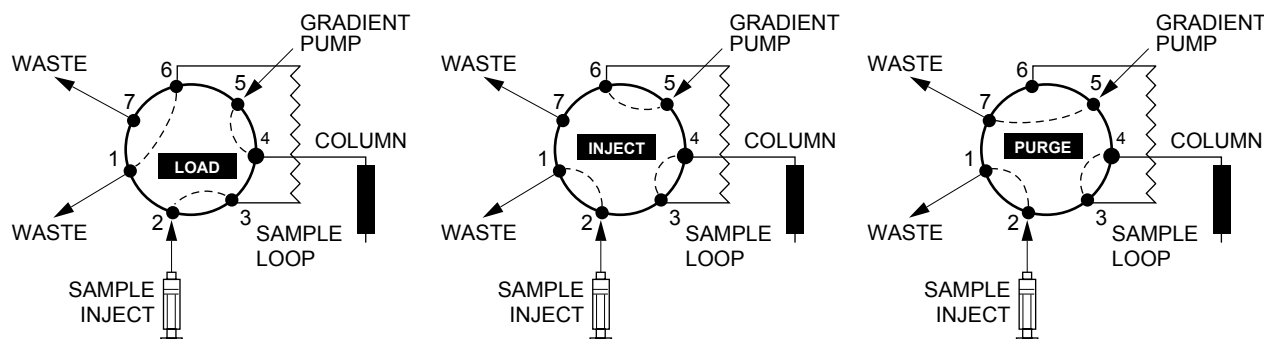
- Make a fitting using 1/16" (1.6 mm) OD tubing and fittings.
- Connect the tubing to the transducer outlet port and to either of the inlet ports on the Mixer, and secure firmly using a twisting action. Plug the unused Mixer inlet port using the plug provided for this purpose. **Ensure a firm connection but do not over-tighten.**

**4. Plumbing from the Mixer to the AVR7-3 Inject Valve.**

- Cut a suitable length of 1/16" (1.6 mm) OD tubing that will reach from the Mixer outlet port to port #5 of the inject valve. Make a fitting using the 1/16" (1.6 mm) OD tubing and fittings.
- Connect the tubing to the Mixer outlet port and to port #5 on the inject valve and secure firmly using a twisting action. **Ensure a firm connection but do not over-tighten.**

## 5. Plumbing the AVR7-3 Inject Valve.

- The inject port assembly and needle are included with each AVR7-3 Inject Valve. Screw this assembly into port #2 of the valve until it is secure. Connect the Sample Loop to ports 3 and 6.
- Plumb the inject valve according to Figure 4-4. Make a fitting using 1/16" (1.6 mm) OD tubing and fittings. Additional information is provided in section 4.4.1 of this chapter and in Section 4, Advanced System Applications.



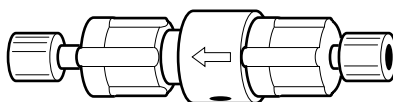
**Figure 4-4. Inject Valve Plumbing for an AVR7-3**

## 6. Plumbing the UV and Conductivity Detectors.

- Connect a piece of 1/16" (1.6 mm) OD PEEK tubing between the column outlet and the bottom of the UV Detector flow cell using 1/4-28 fittings.
- Cut approximately 8 cm of 1/16" (1.6 mm) OD PEEK tubing and attach 1/4-28 fittings and ferrules to each end. Screw one end into the top of the UV Detector flow cell and the other end into the Conductivity Detector flow cell (note that this cell is bi-directional).
- Place the Conductivity flow cell into the notch of the optics bench. This is a gentle push-fit. Note that there is a tag (with a number) attached to the conductivity cable. This number is the flow cell constant and must be entered in the software before beginning a run. (See Table 5-6, **Utilities** drop-down Menu: **Conductivity Flow Cell Constant Calibration**.)
- For flow rates below 10 ml/min, or if the Z flow cell is being used with the UV Detector, insert the 40 psi backpressure device. Plumb the backpressure device following the direction of the arrow.

The backpressure device is required for the following reason: as buffer is pumped through a column, the column exerts a backpressure which serves to keep any air bubbles in solution. Buffer exiting the column returns to atmospheric pressure and air bubbles may "out-gas." As they pass through the flow cell, these bubbles may cause artifacts such as an unstable or spiky baseline. This "outgassing" is minimized by thoroughly degassing buffers and by placing a backpressure device **after** the Conductivity flow cell. This backpressure device puts a small backpressure on the flow cell, keeping the bubbles in solution.

**Note:** When using low pressure columns such as an Econo-Pac® cartridge, plumb the 40 psi backpressure device between the Gradient Pump outlet and the injector valve. This aids in seating the check valves, preventing permanent damage to the cartridge or column.



**Figure 4-5. Backpressure Device**

**7. Connection to a Model 2128 Fraction Collector.**

- a. Make a fitting using 1/16" (1.6 mm) OD tubing and fittings.

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

- b. Connect the fitting between the Conductivity flow cell (or the backpressure device, if that was installed) and the drop-head of the Model 2128 or the Model 2128 diverter valve. (For complete instructions, refer to the documentation for the Model 2128 Fraction Collector.)

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

- a. Connect a piece of 1/16" OD PEEK tubing between the Conductivity flow cell (or the backpressure device, if that was installed) and the SVT3-2 valve common port (labeled "**Common**") using the 1/4-28 fittings.
- b. Connect a piece of 1/16" OD Tefzel tubing from port 1 of the SVT3-2 valve (see Figure 4-9) for use as a waste line.
- c. Connect a piece of 1/16" OD Tefzel tubing from port 2 of the SVT3-2 valve (see Figure 4-9) to the Model 2110 fraction collector drop-head. This tubing sits inside the drop-head, and no fittings are required.

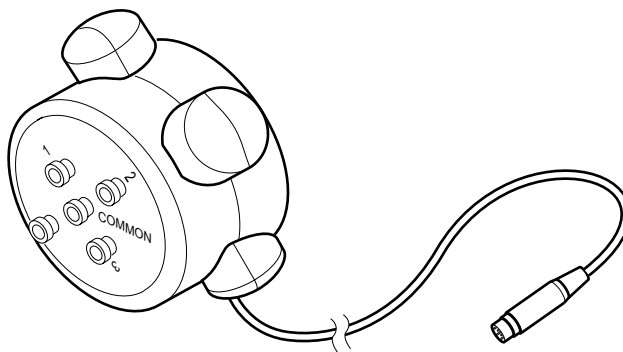
**4.3 PLUMBING BIO-RAD LOW PRESSURE VALVES**

The BioLogic Duo-Flow system may be used to control and monitor the following Bio-Rad low pressure solenoid valves:

- SV5-4 Buffer Select valve
- SVT3-2 Diverter valve

**4.3.1 Plumbing the SV5-4 Buffer Select Valve**

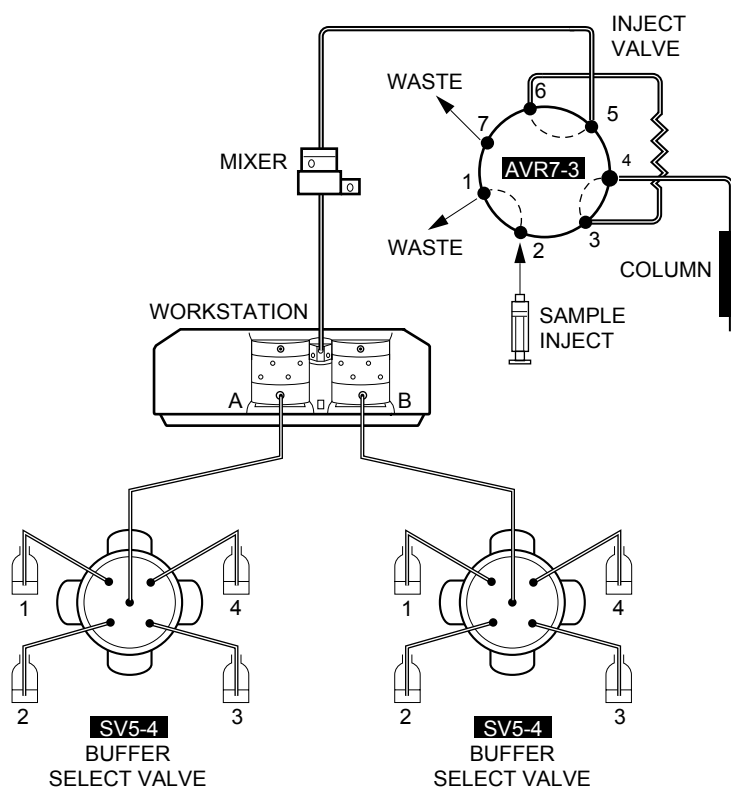
The SV5-4 Buffer Select Valve is a low pressure, 5-port, 4-position valve primarily used for automatic buffer selection when placed before the Gradient Pump. It may also be used for buffer and/or sample selection when placed before an Auxiliary Load Pump, or for fraction collection when placed after the column. This valve is described in detail in its separate documentation. An example of how this valve is used is described in Chapter 8, section 8.1.



**Figure 4-6. SV5-4 Buffer Select Valve**

To install and plumb the valve:

1. When mounting the SV5-4 valve to the System Rack, select a location which will keep tubing lengths to a minimum.
2. Connect the SV5-4 valve cable to any of the available Solenoid Valve connectors on the back of the Workstation (ports 1, 2 or 3).
3. Use 1/8" (3.2 mm) OD PTFE tubing when plumbing the SV5-4 valve as a Gradient Pump inlet valve.



**Figure 4-7. Configuration of the SV5-4 as a Gradient Pump Inlet Buffer Select Valve**

The software's Manual mode lets you manually operate each inlet port on the SV5-4. Manual valve control is useful for the following:

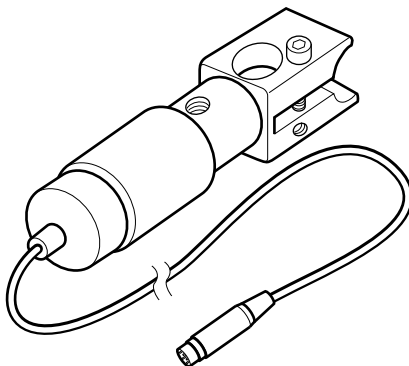
- When priming the tubing and the valves with buffer and or sample prior to starting a method.
- For purging all tubing lines for cleaning purposes.

When programming a Method, the Setup screen lets you define each of the four valve positions by name. This is known as "aliasing," and it allows you to identify the valve by the name or composition of the buffer. The name that you apply in the Setup screen will appear in the Protocol screen when you program an Isocratic Flow step, a Change Valve step, or a Linear Gradient step.

### 4.3.2 Plumbing the SVT3-2 Diverter Valve

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

- To divert the eluant stream from the detectors to a fraction collector or a waste container.
- As a sample select valve when placed before an Pump A.
- As a water rinse valve when placed before Pump B.

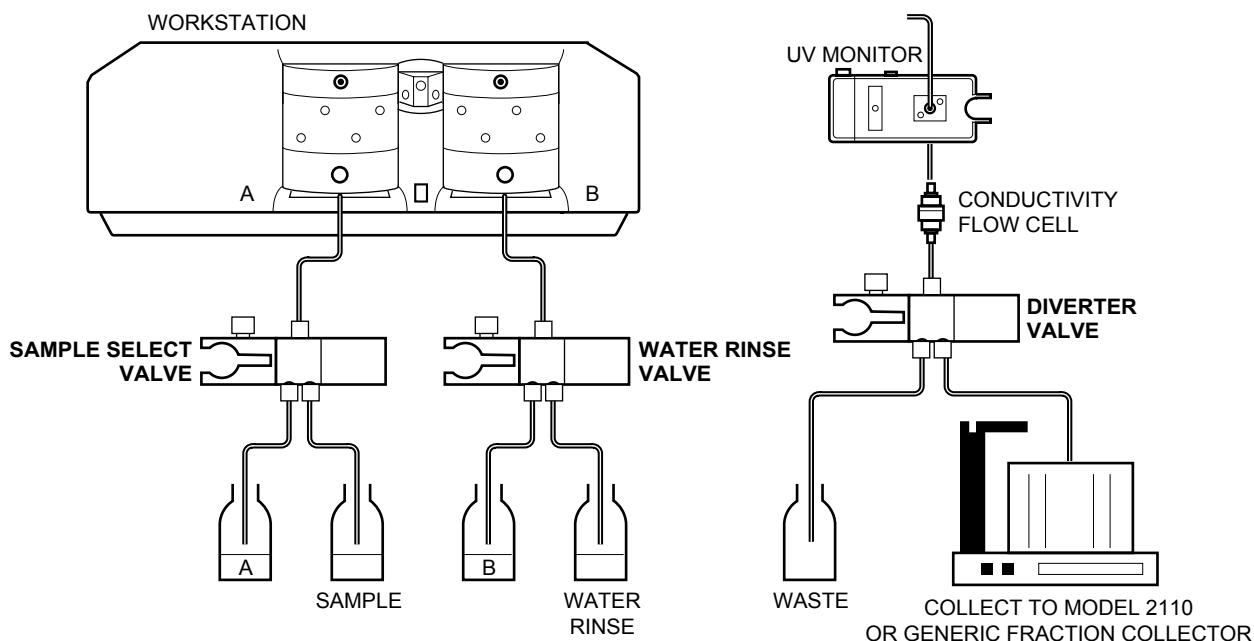


**Figure 4-8. SVT3-2 Diverter Valve**

To install and plumb the valve after mounting it to the System Rack:

1. Connect the SVT3-2 valve's signal cable to any of the available Solenoid Valve connectors on the back of the Workstation (ports 1, 2 or 3).
2. Typically, use 1/16" (1.6 mm) OD PEEK tubing and 1/4-28 fittings when plumbing the valve. Use orange PEEK 0.020" (0.51 mm) ID tubing for the F-10 pumps, and green PEEK 0.030" (0.76 mm) ID tubing for the F-40 pumps.

An exception is when it is used as a Gradient Pump inlet valve; then use 1/8" (3.2 mm) OD tubing.



**Figure 4-9. Configuration of the SVT3-2 as a Fraction Collector Diverter Valve**



The software's Manual mode lets you manually operate each inlet port on the SVT3-2. Manual valve control is useful for the following:

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

The software's **Setup** screen lets you define each of the two valve positions by name. This is known as "aliasing," and it will make programming more convenient. For example, when the SVT3-2 is placed before the Gradient Pump, you can identify the valve by the name or composition of the buffer. The name that you apply in the Setup screen will appear in the Protocol screen when you program an Isocratic Flow step, a Change Valve step, or a Linear Gradient step.

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

#### 4.4 PLUMBING BIO-RAD HIGH PRESSURE VALVES

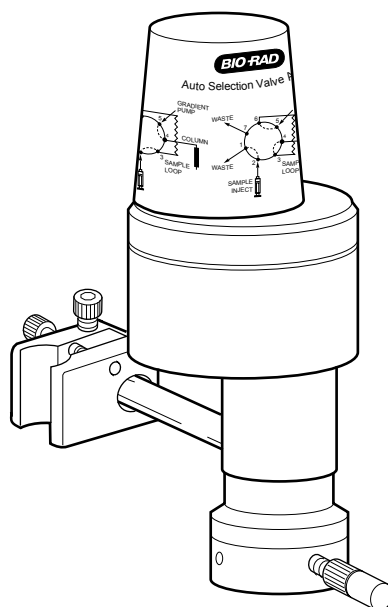
Bio-Rad offers high pressure valves for controlling the flow of sample material. These include the following:

- AVR7-3 automated inject valve
- AVR9-8 automated stream select valve

These valves are discussed in detail in the following sections.

##### 4.4.1 High Pressure Inject Valve (AVR7-3)

The AVR7-3 automated inject valve is a 7-port, 3-position valve, the 3 positions being Load (position 1), Inject (position 2), and Purge (position 3). The Load Position on this valve is the default position when the system is powered up or at the end of a method run unless configured differently from the **Edit User Preferences** window, available from the **Options** menu in the system software.



**Figure 4-10. AVR7-3 Inject Valve**

The AVR7-3 valve has a patented MBB™ (make-before-break) design which provides uninterrupted flow when switching between LOAD and INJECT. This eliminates pump shutdown due to high pressure spikes. This is also beneficial when using fragile columns or flow-sensitive detectors. The valve is rated to 3500 psi and is designed with non-metallic wetted parts and minimal internal dead volume. For additional applications of this valve, see Section 4, Advanced System Applications.

To install and plumb the valve:

**Note:** Do not use an AVR7-3 as a buffer select valve plumbed before the Gradient Pump.

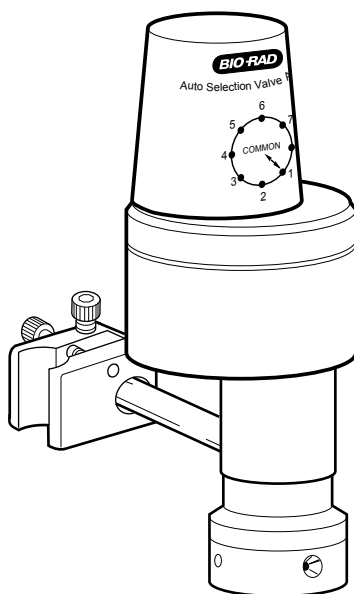
1. Mount the AVR7-3 valve to the System Rack, keeping tubing lengths to a minimum.
2. Connect the AVR7-3 valve cable to any of the available Automated Valve connectors on the back of the Workstation (ports 4, 5, 6).
3. Use 1/4-28, 1/16" (1.6 mm) OD fittings and tubing when plumbing this valve. Use orange PEEK 0.020" (0.51 mm) ID tubing for flow rates of 10 ml/min or less, and green PEEK 0.030" (0.76 mm) ID tubing for flow rates greater than 10 ml/min. See Figure 4-4 for a diagram of this plumbing. Keep tubing as short as possible.

#### 4.4.2 High Pressure Stream Select Valve (AVR9-8)

The AVR9-8 automated valve is bi-directional, with port 9 being the common port. Position 1 on the AVR9-8 is the default position when the system is powered up or at the end of a Method run unless configured differently from the **Edit User Preferences** window, available from the **Options** menu. The valve is rated at 3500 psi and is designed with non-metallic wetted parts for bio-compatibility and minimal internal dead volumes.

The stream-select valve has a number of uses in a chromatography system. (Further information is provided in Section 4, Advanced System Applications, Chapters 8 - 10.) The valve's use dictates its plumbing. For example:

- When two AVR9-8 valves are used, up to eight columns may be run sequentially.
- When used in conjunction with an Auxiliary pump (such as the Bio-Rad Model EP-1 Econo Pump) for loop filling and an AVR7-3 Inject valve, the AVR9-8 automated valve may be used to select up to eight samples for consecutive chromatography runs.



**Figure 4-11. AVR9-8 High Pressure Stream Select Valve**

To install and plumb the valve:

1. Mount the AVR9-8 valve to the System Rack. Select a location which will keep tubing lengths to a minimum.
2. Connect the AVR9-8 valve Signal Cable to any of the available Automated Valve connectors on the back of the Workstation (ports 4, 5 or 6).
3. Use 1/4-28 fittings and 1/16" (1.6 mm) OD PEEK tubing when plumbing these valves. Use orange PEEK 0.020" (0.51 mm) ID tubing for the F-10 pumps, and green PEEK 0.030" (0.76 mm) ID tubing for the F-40 pumps.

## 4.5 PRIMING THE SYSTEM

Before a method can be run, the system must be purged of air. Prior to running aqueous solutions through the system, pump 100 ml of methanol (MeOH) through each pumphead at 5 ml/min with the 40-psi backpressure device in place. Once this is done, prime the remainder of the system with water.

### 1. Priming the Workstation Gradient Pump.

- Immerse the Gradient Pump A and B inlet lines in a container of HPLC grade (filtered, degassed) or other high quality water.
- Place a 10 ml luer syringe (supplied with the fittings kit) on the priming port of pump head A.
- Turn the priming port counter-clockwise to open the seal and gently withdraw the syringe plunger to draw water into the pump head.
- Repeat this operation until no air bubbles are visible in the inlet tubing.
- Tighten the priming port by turning it clockwise.
- Repeat this priming procedure for pump head B. **See Section 11.3.1 for additional priming procedures.**

### 2. Moving the Inject Valve to Purge Position.

- Go to the **Manual** screen displayed by the system software.  
If you plugged an inject valve into port #4, you will see a valve box designated “**AVR7-3 at port 4.**” The three radio buttons of this box correspond to valve positions as follows: **L** = Load position, **I** = Inject position, **P** = Purge position.
- To move the AVR7-3 valve to Purge position, click button **P**.  
**Note:** The default position at power up and at the end of a programmed method for the AVR7-3 is position **L**, and for all other automated valves is position 1 unless changed from the **Edit User Preferences** window, available from the **Options** menu.

### 3. Purging the Gradient Pump.

With the inject valve in position **P** (Purge):

- Press the Purge buttons A and B on the front of the Workstation. The Gradient pump will run and the indicator lights will flash green. The default purge flow rate for the F-10 pumps is 10ml/min; that for the F-40 pumps is 40 ml/min. (This may be changed using the **Manual Setup** window, available from the **Options** menu.)
- Run both pumps for about 2 minutes and then press the Purge buttons again to stop the pump.

### 4. Flushing the system through to the fraction collector.

With the Gradient Pump stopped, move the inject valve back to position **I** (Inject) by clicking the radio button **I** (AVR7-3) on the **Manual** screen.

Using the **Manual** screen, set the pump flow rate at 1.0 ml/min and start the pump. Water will now flow through the sample loop, the UV and Conductivity flow cells, and ultimately to the fraction collector.

**Note:** If the system pressure variations exceed  $\pm 10\%$ , refer to section 11.3.1, Priming the Pump and Removing Trapped Air Bubbles.

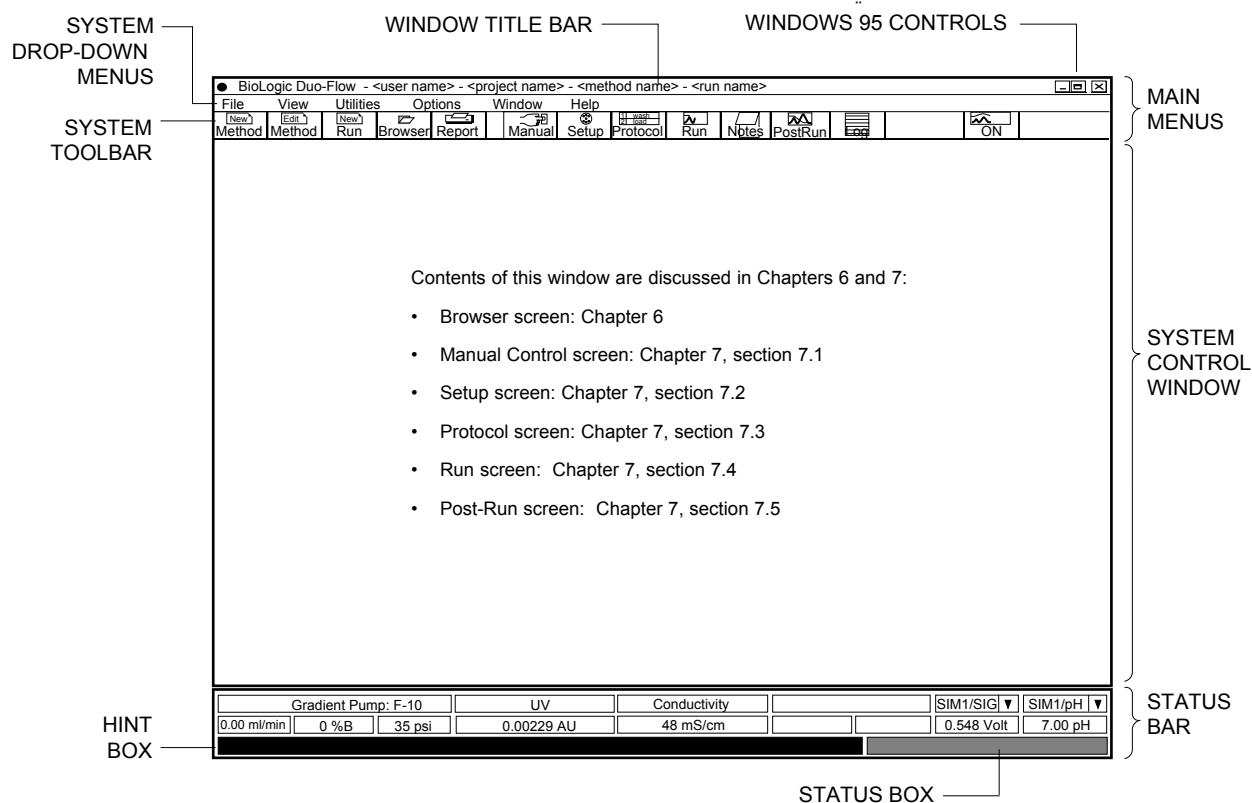


## 5.0 INTRODUCTION TO THE SYSTEM SOFTWARE

The BioLogic Duo-Flow system software is based on Microsoft Windows® 95, a widely used operating system for PC-compatible computers. This chapter discusses how to use the BioLogic Duo-Flow system software.

### 5.1 USING THE SYSTEM INTERFACE

When you first turn on the system, the Manual Control screen is displayed (see Figure 5-1). Note that this screen, like all screens in the interface, is grouped into three sections, which consist of the Main (or System) Menus, System Control Window, and System Status Bar.



**Figure 5-1. Layout of the Screen Display**

The BioLogic Duo-Flow system is controlled and monitored using the following:





- **System Toolbar:** This toolbar is the primary navigation tool for the system software.
- **System drop-down menus:** These menus are always displayed. Access to advanced functions are found only in the drop-down menus. **Note:** Some functions found on the System Toolbar are duplicated in the drop-down menus.
- **System Status bar:** This area shows information about components in the system, as well as providing information about software icons.

**Note:** Items shown in gray are not currently active or cannot be accessed because they are inappropriate to the current mode.

## 5.2 STANDARD MOUSE AND KEYBOARD FUNCTIONS

The BioLogic Duo-Flow system is supplied with a standard PC-compatible mouse and keyboard. The left mouse button is used with system software, except as noted.

**Table 5-1.**  
**Special Function Keys**

Special Function Keys	Description
	<b>Hold until Keypress:</b> This is used during a run to advance the Method (i.e., satisfy the Hold) when the Method includes a “Hold until Keypress” step.
	<b>Help:</b> Displays the Help menu for the currently displayed screen.
	<b>Esc:</b> Functions as an alternative to the Cancel selection in a Dialog box.
	<b>Alt:</b> Some system commands can be executed either by selecting them from a drop-down menu or by holding down the <b>Alt</b> key and then pressing the appropriate character key.



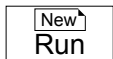


### 5.3 SYSTEM MENUS

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

#### 5.3.1 System Toolbar Buttons



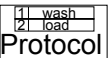




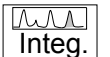
Most of the System Toolbar buttons are continuously displayed across the screen. The function of each toolbar button is provided in Table 5-2.

**Table 5-2.**  
**Toolbar Buttons**

Button	Description
<b>File Buttons</b>	<b>Note:</b> Similar functions can be found by selecting the File drop-down menu.
	Initiates the process of writing a new method. The new method will be saved into the current User and Project directories. The current user and project is shown in the window title bar. <b>Note:</b> To place a new method in a different user and project, choose <b>Select User and Project</b> under the File menu before clicking on the <b>New Method</b> button in the Toolbar. Choose the desired user and project from the drop-down list. To create a new user and project, you must use the Browser.
	This button is available when an open method has runs associated with it. It allows you to create a new method based on the open method. A copy of the open method is made, and the system software prompts you for a name change. <b>Note:</b> The original method remains unaltered.
	Initiates a new run for the currently open method. You will be prompted for a new run name prior to launching the run.
	Opens the database of existing methods and runs. See chapter 6 for a complete description of the browser.
	Allows you to define when to print and what is to be printed in the report for a run.



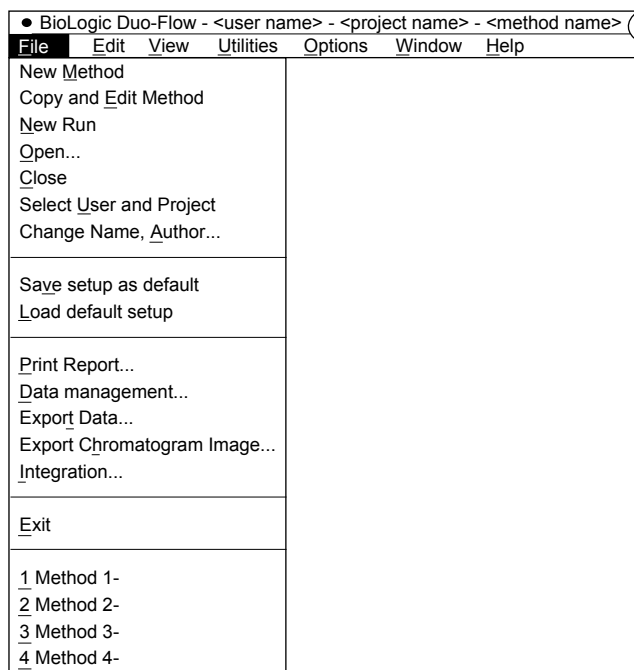
**Table 5-2. (continued)**  
**Toolbar Buttons**

Button	Description
<p><b>View Buttons</b></p> <div data-bbox="203 485 311 541">Manual</div> <div data-bbox="203 646 311 703">Setup</div> <div data-bbox="203 814 311 871">Protocol</div> <div data-bbox="203 919 311 976">Run</div> <div data-bbox="203 1060 311 1117">Notes</div> <div data-bbox="203 1207 311 1264">PostRun</div> <div data-bbox="203 1312 311 1369">Log</div> <div data-bbox="203 1432 311 1488">Integ.</div>	<p><b>Note:</b> Similar functions can be found by selecting the View drop-down menu.</p> <p>Displays the Manual screen, which allows individual control of instruments and devices in the system. In Manual mode, data is viewed from the electronic chart recorder; data cannot be saved.</p> <p>Displays the Setup screen, which allows you to choose components required for your method. From the Setup screen you can “alias” (add descriptions to) valve positions and buffers. The Setup screen is automatically displayed when you write a new method.</p> <p>Displays the Protocol screen for creating and editing a method.</p> <p>Displays the Run screen, from which you can initiate a run of the currently loaded method. You will be prompted for a new run name prior to launching the run.</p> <p>The notes section is a prompted section which allows you to enter additional information about your method and/or run. This section is always editable, even after a run is executed.</p> <p>PostRun allows you to customize your chromatogram prior to printing.</p> <p>As the run progresses, all events are recorded in the Log. This information cannot be edited.</p> <p>Launches the EZLogic integration software, which is a software option. This button replaces Log when EZLogic is installed.</p>

### 5.3.2 System Drop-down Menus

The system drop-down menus are discussed in the following tables.

**Table 5-3.**  
**File Drop-down Menu**



The File menu is always displayed. It consists of the following:

- **New Method:** Initiates the process of writing a new method. The new method will be saved into the open User and Project directories. To save to a different User or Project, first select **Select User and Project**. Selecting **New Method** during a run will automatically open the “BioLogic offline” function, allowing you to create a method while a run is in progress.
- **Copy and Edit Method:** Allows you to use an existing method as a template for a new method. A copy of the method is made, and the system software prompts you for a name change.
- **New Run:** Initiates a run for the currently open method. You will be prompted for a new run name prior to launching the run.
- **Open:** Displays the browser. Refer to Chapter 6, Browser Screen.
- **Close:** Closes the current method and/or run.
- **Select User and Project...:** Allows you to select the default user and project of the system when new methods are created.
- **Change Name, Author...:** Allows you to change the method/run name, author, and description. This function is inactive once a run has been initiated.
- **Save setup as default:** When you create a method, the Setup screen displays a list of devices in setup. This is the default list of devices. If you have a method with a setup list that more accurately reflects your use of the system, you can open this method and then select **Save setup as default**. Then each time you create a method, the Setup screen will display this list of devices in setup.
- **Load default setup:** Recalls and loads the default setup.

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

- 
- **Print Report:** Initiates the generation and printing of the currently open method, including its setup and run data, run results, and the run log report.
  - **Data Management:** Displays the browser. Refer to Chapter 6, Browser Screen.
  - **Export Data:** This feature establishes data export parameters, and is available from the PostRun screen.
  - **Export Chromatogram Image:** This feature exports a chromatogram image in a Windows Meta File (.WMF) format. This feature is available in the PostRun screen.
  - **Integration:** Launches the EZLogic integration software. Contact Bio-Rad for more information on EZLogic.
  - **Exit:** Exits the BioLogic application.
-

**Table 5-4.**  
**Edit Drop-down Menu**

Edit menu: Setup Screen		Edit menu: Protocol Screen	
● BioLogic Duo-Flow - <user name> - <project name> - <method name> File Edit View Utilities Options Window Help Delete Select All		● BioLogic Duo-Flow - <user name> - <project name> - <method name> File Edit View Utilities Options Window Help Cut Copy Paste Delete Select All:	
Edit menu: Run Screen		Edit menu: Post Run Screen	
● BioLogic Duo-Flow - <user name> - <project name> - <method name> File Edit View Utilities Options Window Help Multiple runs... Start run Pause buffer pump Hold gradient		● BioLogic Duo-Flow - <user name> - <project name> - <method name> File Edit View Utilities Options Window Help Tag and Trace Options Full view in zoom chromatogram Print zoom chromatogram Copy zoom chromatogram to clipboard Delete Selected tag: Delete all tags	

The contents of the Edit menu depends upon the currently displayed screen. In most instances, the item in the drop-down menu also appears in the System Toolbar; exceptions are noted below.

### Setup Screen

- **Delete:** Deletes the currently highlighted device in the Devices in Setup window of Setup screen. To delete all devices, first select Select All, as described below.
- **Select All:** Highlights all devices in the Devices in Setup window of the Setup screen. (Not available from the System Toolbar.)

### Protocol Screen

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

### Run Screen

- **Multiple runs...:** Specifies the number of times the method is to run.
- **Start run:** Initiates the run.
- **Pause buffer pump:** Pauses a run and stops the pumps.
- **Hold gradient:** Holds a run at the current %B conditions and flow rate. It also holds method time, effectively holding the protocol step and the current fraction collection step if collection is programmed.

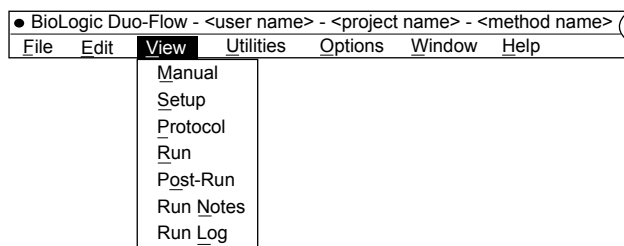
*Table 5-4. (continued)  
Edit Drop-down Menu*

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**Post Run Screen**

- **Tag and Trace Options...:** Displays the Post-Run Tags window that allows you to specify which traces are to be displayed, whether or not their tags are to be displayed, the tag style for the active trace, and the values for each tag in the active trace. (Also available from the **Tag** button in the System Toolbar.)
  - **Full View in zoom chromatogram:** Allows you to view the complete chromatogram. (Also available from the **Full View** button in the System Toolbar.)
  - **Print zoom chromatogram:** Allows you to print the complete chromatogram. (Also available from the **Print** button in the System Toolbar.)
  - **Copy zoom chromatogram to clipboard:** Copies to clipboard the complete chromatogram. From the clipboard, it can then be copied into other applications. (Not available from the System Toolbar.)
  - **Delete selected tag:** To delete a tag, highlight the selected tag before using this function. (Also available from the **Del. Tag** button in the System Toolbar.)
  - **Delete all tags:** Deletes all tags in the chromatogram. (Not available from the System Toolbar.)
-

**Table 5-5.**  
**View Drop-down Menu**



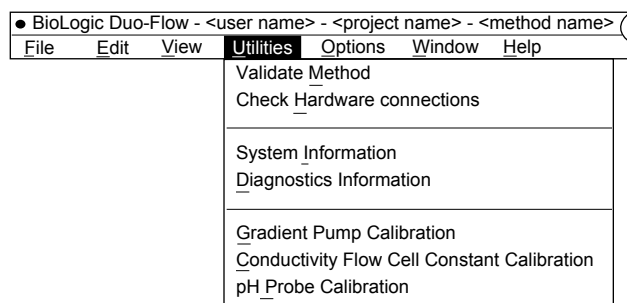
Each of these selections is available from the toolbar menu. The View menu consists of the following:

- **Manual:** Displays the Manual screen for individual control of installed instruments and devices in the system.
- **Setup:** Displays the Setup screen, which allows you specify the components required for your method.
- **Protocol:** Displays the Protocol screen for creating and editing chromatography steps in the method.
- **Run:** Displays the Run screen, from which you can initiate the running of the currently open method.
- **Post-Run:** When a run is completed, select **Post-Run** to annotate the chromatogram (for example, to apply tags to UV, Conductivity, and/or %B values).
- **Run Notes:** To keep notes regarding the run, select **Run Notes** and enter them into the Notes screen. There are fields for description of the sample, the column, the operator, the buffer(s), flow rates, gradients, chart speed, fraction size, and general text entry of variable length. These notes are printed with the report.
- **Run Log:** Displays a log of all events occurring during the run. This information cannot be edited. **Note:** The Run Log may be disabled by selecting **Edit User Preferences** from the Option drop-down menu. This will save hard drive space. However, it is recommended that the Run Log be active for assistance in troubleshooting.

**Note:** If the Integration software option is installed, the **Log** toolbar button is replaced by the Integ toolbar button. To display the Log window, select **Run Log** from the View drop-down menu.

---

**Table 5-6.**  
**Utilities Drop-down Menu**



These selections generally relate to system options, and it remains the same regardless of which screen is displayed. The Utilities menu consists of the following:

- **Validate Method:** This verifies that the devices required by the Protocol have been defined in the Setup screen. Validation is done automatically at the initiation of a new run.
  - **Check Hardware connections:** This checks that all devices appearing in the Setup screen and required for the run are connected to the system. It does not apply to a V7-3 Manual Inject valve, chart recorder, Model 2110 or generic fraction collector, or Auxiliary pump. It does not verify plumbing connections.
  - **System Information:** Displays the current system configuration, including each instrument in the system and its firmware version number, the Windows® version number, available hard disk space, and the number of methods and runs in the database.
  - **Diagnostics Information:** Displays information for service diagnostic purposes only.
  - **Calibrate Gradient Pump:** This selection is available only from the Manual screen, and it is typically used only after servicing of the pump. It allows the user to calibrate the gradient pump flow rate and zero the system pressure gauge. **Note:** You must exit the Calibration screen in order for the system to accept the calibration values.
  - **Conductivity Flow Cell Constant Calibration:** This utility allows the user to calibrate the conductivity flow cell. The conductivity flow cell constant is noted on the flow cell cable. When a new conductivity flow cell is used, use this selection to enter the flow cell constant. **Note:** You must exit the calibration screen in order for the system to accept the calibration values.
  - **pH Probe Calibration:** This utility allows the user to calibrate the pH probe. It is typically used at the start of each day's use of the system. **Note:** You must exit the calibration screen in order for the system to accept the calibration values.
-

**Table 5-7.**  
**Options Drop-down Menu**

---

● BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File	Edit	View	Utilities	Options	Window	Help
				Manual Setup		
				Edit User Preferences		
				Chromatogram Settings		
				Status lines		
				Browser Settings		
				View Chromatogram		

---

These selections generally relate to screen options.

- **Manual Setup:** From this screen you can set the pump's purge flow rate and the SIM-HR parameters. For the F-10 pumps, you can specify a maximum purge rate of 10 ml/min; for the F-40 pumps, you can specify a maximum purge rate of 40 ml/min.
- **Edit User Preferences:** This lets you specify Run Options (which determine whether or not the protocol's steps are listed in the Run Log and whether or not valves are automatically returned to position 1 at the end of the run) and the protocol program base (time or volume).
- **Chromatogram settings:** Allows you to select which traces will be visible in the chromatograms. For the Manual screen, the X-axis and the Y-axis ranges can be set. For the Run and Post-Run screens, only the Y-axis range can be set.
- **Status Lines:** Allows the status message at the bottom left of the screen to be toggled on or off.
- **Browser settings:** Displays the Browser & Database Options window. The Browser options allow you to select how the browser is to display users/projects/methods/runs. For example, select Projects to display a top level folder called "Projects" which contains all projects in the database.

The Options for Database selections typically should not be changed. They allow the system to test and optimize the database upon startup of the system. These settings check the integrity of the data and reduce the size of the database file.

- **View Chromatogram:** Allows the Manual screen chromatogram to be toggled on or off. When off, the valve panel is displayed.
-



**Table 5-8.**  
**Window Drop-down Menu**

---

● BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File	Edit	View	Utilities	Options	Window	Help
					1 BioLogic Online - <method name>	
					2 BioLogic Offline - <method name>	

---

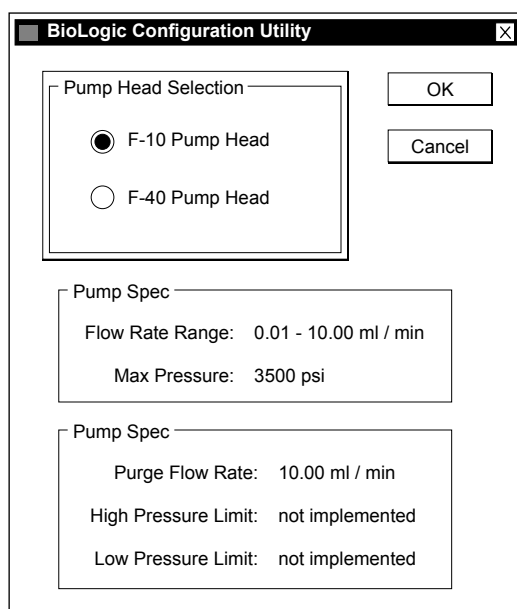
The BioLogic Duo-Flow software allows you to work offline while a run is in progress. A complete discussion of this function is provided in section 7.4.2, Working Offline.

- **BioLogic Online:** Allows you to manipulate all screens to complete a run.
  - **BioLogic Offline:** Allows you to write a protocol, analyze data, and export data. A run cannot be started while in offline mode.
- 

## 5.4 BIOLOGIC CONFIGURATION UTILITY SOFTWARE

Whenever the pumps are changed, the BioLogic Configuration Utility software must be run before the system is used. The BioLogic Configuration software updates the flow rate and pressure ranges in the BioLogic Duo-Flow software. To run this software:

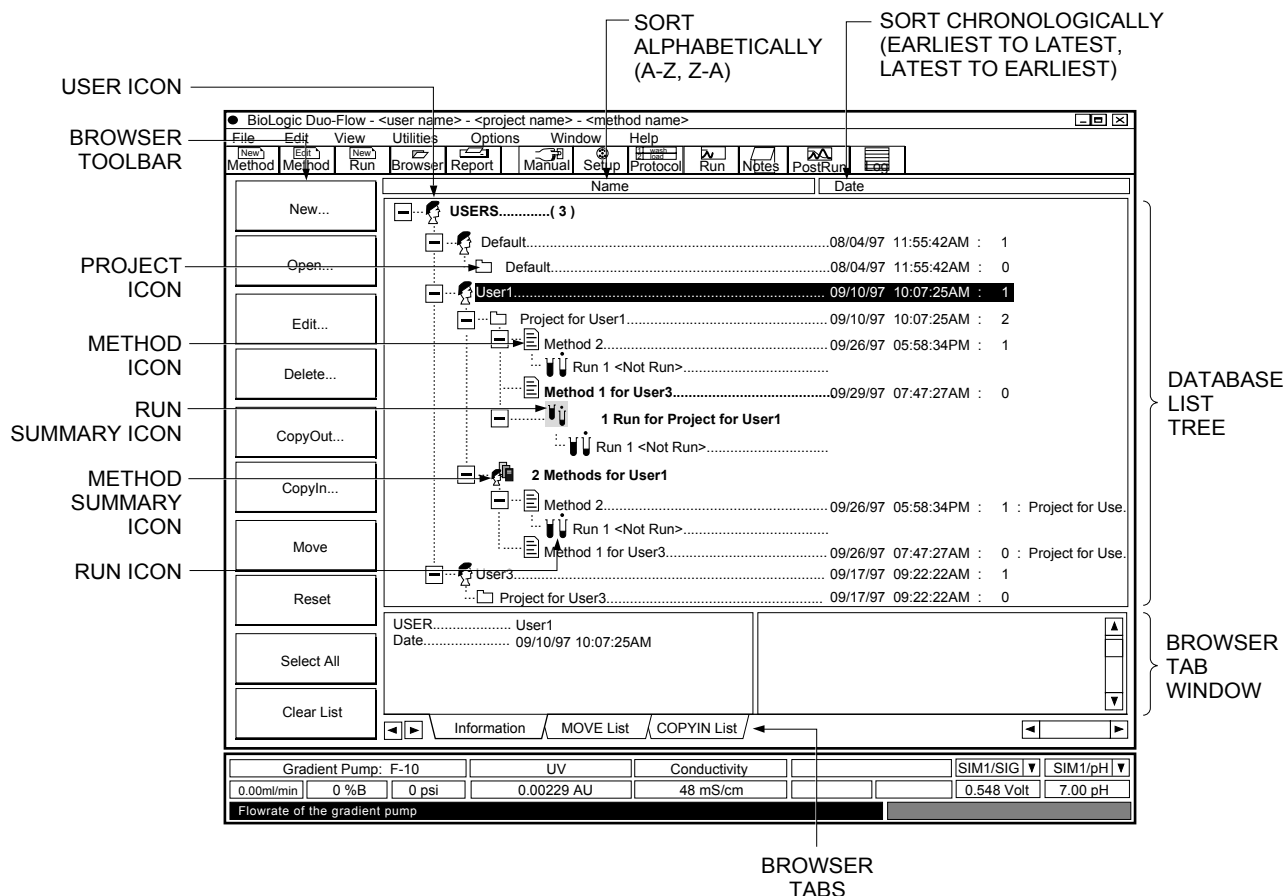
1. Exit the BioLogic Duo-Flow software by selecting **Exit** from the File drop-down menu.
2. Double-click on the BioLogic Configuration icon.
3. In the BioLogic Configuration Utility window that appears, indicate the pump which will be used with the system.
4. Exit the BioLogic Configuration Utility and double-click on the BioLogic Duo-Flow icon to start that software.



**Figure 5-2. BioLogic Configuration Utility Software Screen**

## 6.0 INTRODUCTION TO THE BROWSER SCREEN

The browser is the organizational tool for chromatography data. It is a database that is displayed as a tree hierarchy which can be sorted by Users, Projects, Methods, and Runs. Figure 6-1 shows the layout of the Browser screen.



**Figure 6-1. The Browser Screen**

The Browser screen displays the following information and controls:

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



Click on this icon in the database tree to collapse the listing for a user, project, method, or run folder.



Click on this icon in the database tree to expand the listing for a user, project, method, or run folder.

RESET

Click on this button in the Browser toolbar to collapse the tree to its top level items.

- **Icons displayed in green or red in the database tree indicate the following:**

Green: Currently open in the active window. The active window may be the on-line window or the off-line window. For more on on-line and off-line, refer to the discussion in Chapter 7, section 7.4.2, Working Off-Line.

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

- **Icon colors other than red and green in the database tree are as follows:**

Blue (profile): User folder

Yellow: Project folder

Blue (text page): Method folder

Blue (test tubes): Runs

- **Use of bolded text in the database tree applies to the following:**

Top level folders: Typically this applies to the USERS folder, which lists all users of the system. However, by selecting **Browser Settings** from the Options menu, you can use the "Starting Browser Selections" to move Projects, Methods, and Runs to the top level of the tree hierarchy.

Summary information: The total number of Methods are listed for each user, and the total number of Runs are provided for each user project folder. **Note:** You cannot delete summary information.

In addition to the database tree, the Browser screen displays the following controls and information:

- **Browser toolbar:** These buttons control the different uses of the Browser screen. They are discussed in greater detail later in this chapter.
- **Name and Date buttons:** These buttons, located across the top of the tree hierarchy, allow you to sort the tree alphabetically or chronologically. These are toggle buttons. For example, if you click on the Name button to sort from a to z, then clicking again sorts from z to a. If you click on the Date button to sort from the latest listing, then clicking again sorts from the earliest listing.
- **Browser Tabs and Browser Tab window:** The Browser Tabs are listed at the bottom of the Browser screen, and they control what is displayed in the Browser Tab window. The following tabs are available:

Information: Clicking on this tab displays two panels in the Browser Tab window: The left panel shows details about the specific item selected; the right panel displays information about the next higher level item to which the item belongs.

MOVE List: Displays the list of all items selected to be moved from one place to another in the database list. Items selected to be moved are indicated by a red checkmark in the database tree. Projects can be moved from one user to another; Methods can be moved from one Project to another. When a Project is moved, it takes all Methods and Runs associated with it; when a Method is moved, it takes all Runs associated with it. The procedure for moving an item is discussed later in this chapter.

COPYIN List: Displays the list of all items selected to be copied in (restored) to the database from an archived data file. The procedure for copying in items is discussed later in this chapter.

The following steps provide procedures for creating a run and using the Browser.

### 1. Define a New User.

The first step is to define a new user. This allows you to group your methods and runs within the Browser. To define a new user,

- a. Click once on a User icon in the Browser.
- b. Click once on the **New...** button and select New User. You will be prompted for a new User Name. Enter the name you would like to use.
- c. Press **OK** to accept the new name.

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

**Note:** If you define a new user, you must also assign a project to the user before writing a new method.

### 2. Define a New Project.

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

- a. With the new User Name highlighted, click once on the **New...** button.
- b. Select **New Project**. Enter the project name and description.
- c. Press **OK** to accept the new name.

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

### 3. Write a New Method.

To begin writing a new method from the Browser:

- a. Select the Project folder or a Method in the Project folder where you want the new Method to be located.
- b. Click once on the **New...** button.
- c. Select **New Method**. Enter the method name and press **OK**.

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

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

### 4. Name a New Run.

To start a new run from the Browser:

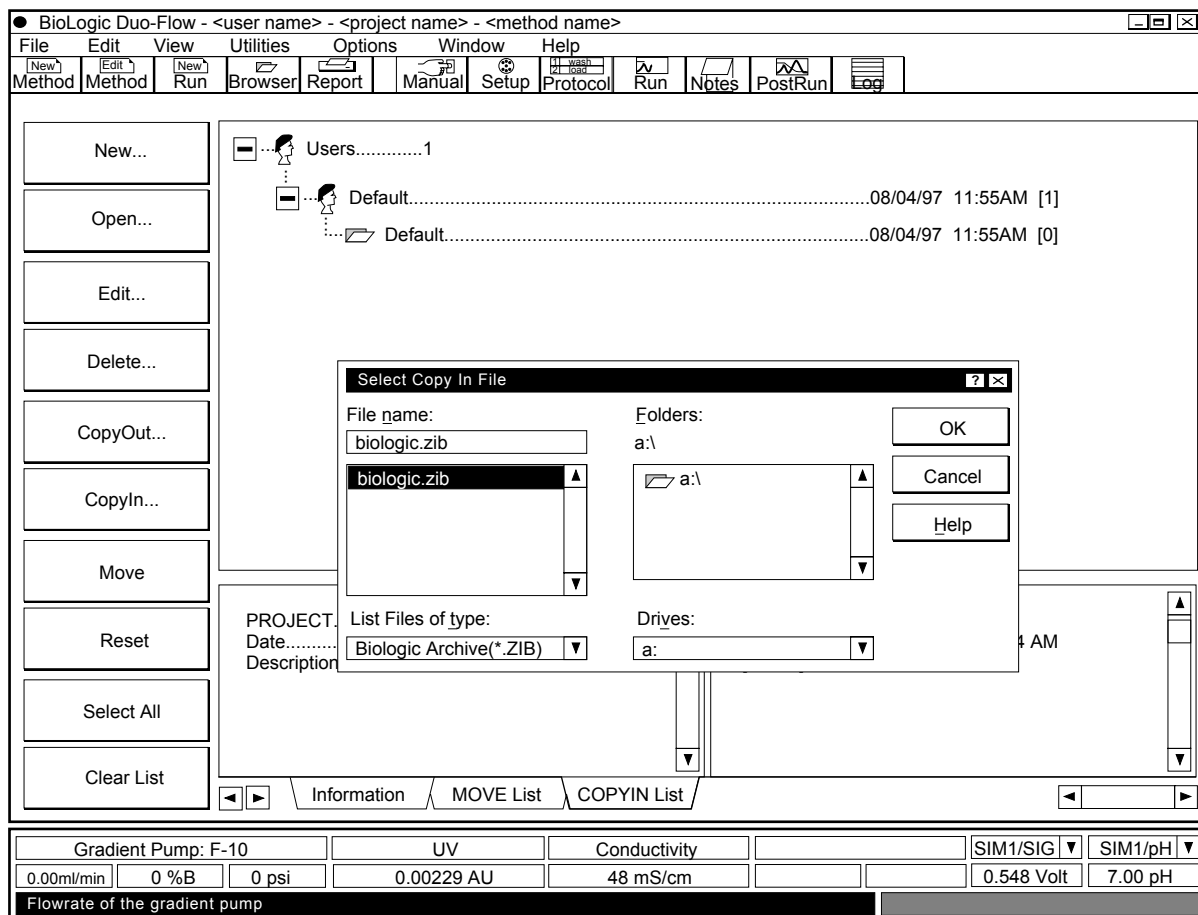
- a. Select the Method folder or a Run in the Method folder where you want the new Run to be located.
- b. Click once on the **New...** button.
- c. Select **New Run**. Enter the run name and press **OK**.

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

2. You can also use the **New Run** button in the System Toolbar. By default the system assumes that the current user, project, and method shown in the window title bar is the path for the new run.

The Browser toolbar buttons are described below:

- New...** The **New...** button creates a new User, Project, Method, or Run, depending on which icon within the Browser is highlighted.
- Open...** The **Open...** button allows you to open a selected method or run from the database tree. Opening a Run allows you to view, analyze, and print the run data.  
**Note:** Opening a Method or Run while a run is in progress will result in the Run-in-progress remaining in the On-Line window and the selected method or run being opened in the Off-Line window. For more on On-Line and Off-Line, refer to the discussion in Chapter 7, section 7.4.2, Working Off-Line.
- Edit...** There are multiple functions of the **Edit...** button, depending upon what is highlighted when the button is pressed. Selecting a user or project name allows you to change that user name or project name and/or description. Selecting a Method or Run copies the contents of the selected item. When a method is selected, you create a new method when you enter the method name, author, and description. When a run is selected, you can change the run name, operator, and description. **Note:** Summary items are not editable.
- Delete** The **Delete** button eliminates Users, Projects, Methods and Runs from the Browser. This is useful after using the Copy Out function to archive files to floppy disk, as discussed below. In order to delete an element of the Browser, you must first delete data associated with the element. For example, to delete a Method, you must first delete all of the Runs associated with the Method.  
**Note:** You cannot delete Methods or Runs from method and run summaries. Also, after deleting files, you may want to run the Windows 95 disk defragmenter. This is available from the Windows **Start** button: select **Programs** → **Accessories** → **System Tools** → **Disk Defragmenter**.
- CopyOut...** This function lets you copy methods and runs to floppy disk or to another location on your computer's hard disk. The copy out function is used for backup and archiving purposes and to transfer data to another BioLogic Duo-Flow system.  
Copied out files are stored in a file with the default name biologic.zib, which is a compressed file format to conserve disk space. The copy out function copies the User, Project, and Method names associated with the Run. **Note:** Initiating a CopyOut to floppy disk completely erases the contents of the floppy disk. Do not use a floppy disk containing data that you want to keep.  
To copy out, highlight the Runs you wish to copy out. Press the **CopyOut...** button on the Browser Toolbar. A dialog box appears which allows you to select the destination of the .ZIB file (either the hard drive or a floppy disk). Choose the destination and press **OK**.



**Figure 6-2. The Copy In Window**

**CopyIn...** This function allows you to copy in methods and runs from a backed-up or archived .ZIB file. To copy in,

- Press the **CopyIn...** button and select the .ZIB file you wish to CopyIn. (See Figure 6-2.)
- From the COPYIN List in the Browser Tab window, select the desired methods and runs.

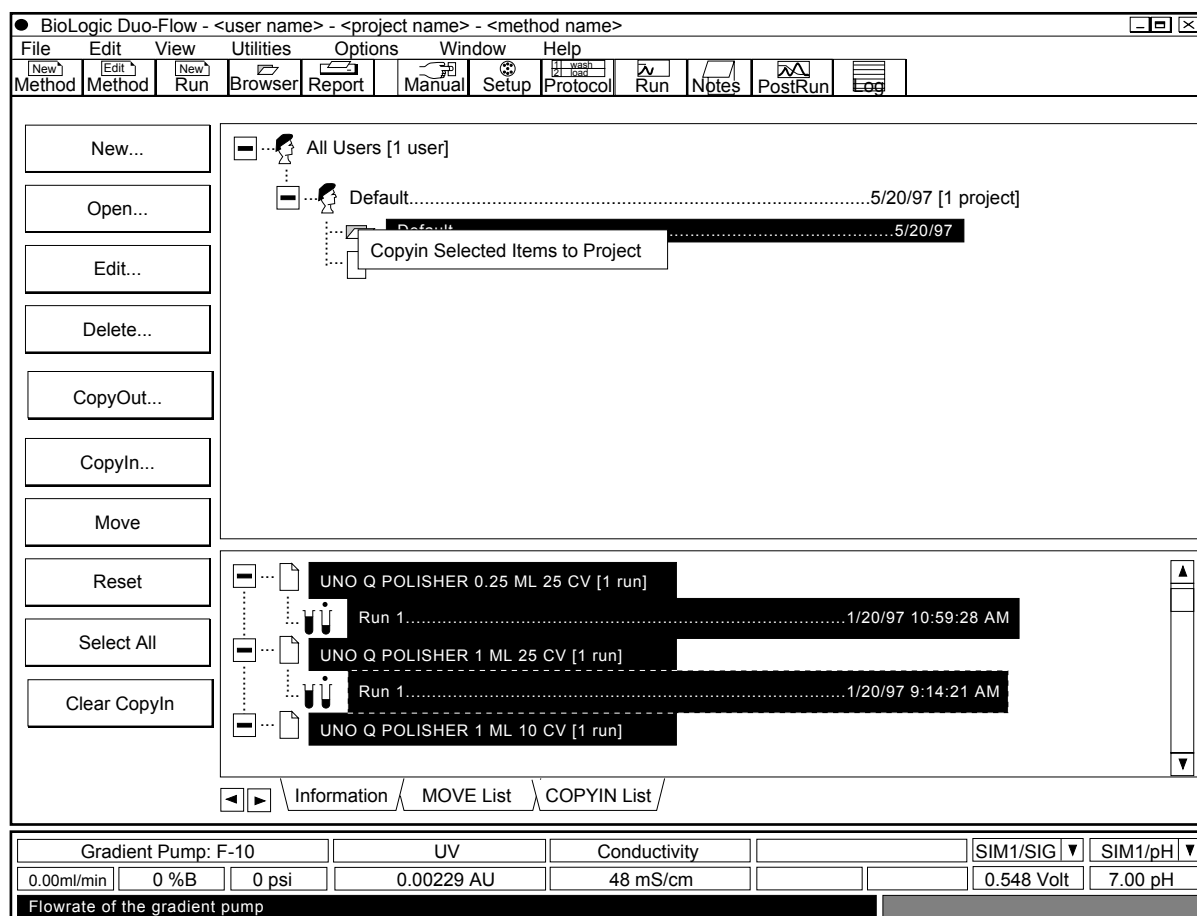
All methods and runs in the file are displayed in the COPYIN List. (See Figure 6-3.)

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

- Again, click on the **CopyIn...** button (or click on the right mouse button) and select **Copy to <project name>**.

**Note:** Clicking the right mouse button displays all the available Browser toolbar buttons for the *highlighted* selection.

- Once you finish the copy in procedure, use the **Clear List** button on the Browser toolbar to delete the remaining methods and runs in the COPYIN List.



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

## Move

Move allows you to transfer a selected item from one location to another. For example, a Project can be moved from one user to another, and a Method (and its Runs) can be moved from one Project to another. Individual Runs cannot be moved. To move an item,

- In the database tree, highlight the item you want to move. You may highlight a Project or a Method.
- Click once on the **Move** button on the Browser Toolbar. The items to be moved will appear in the MOVE List in the Browser Tab window.
- From the MOVE List, select what you want to move.
- From the menu tree, select the new destination for the items you are moving.
- Again click on the **Move** button. The items will automatically move to the destination.  
**Hint:** Alternatively, highlight the destination icon, right-mouse click and select the "Move Selected" message with a left-mouse click.
- Once you finish the move procedure, use the **Clear List** button on the Browser toolbar to delete the remaining methods and runs in the MOVE List.

## Reset

Collapses all of the folders in the Browser.

## Select All

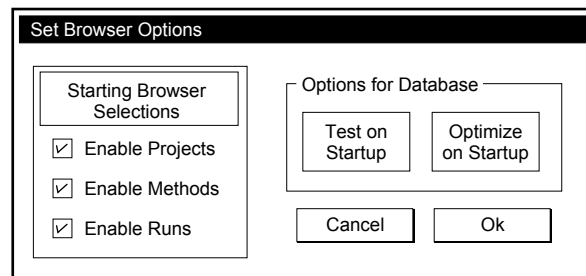
This button is active during the CopyIn and Move commands. It selects all contents of the Browser Tab window.

## Clear List

Clears the listing in the Browser Tab window.

There are several Browser options available by selecting **Browser Settings** from the **Options** menu, discussed in Table 5-7 Options Drop-down Menu. The **Set Browser Options** window that is displayed provides the following options:

- **Enable Projects:** A summary of all Projects, regardless of User, will be listed in the Browser.
- **Enable Methods:** A summary of all Methods, regardless of User or Projects, will be listed in the Browser.
- **Enable Runs:** A summary of all Runs will be listed in the Browser.
- **Test on Startup:** Upon software startup, the Browse database is automatically tested.
- **Optimize on Startup:** Upon software startup, the Browse database is automatically optimized for maximum efficiency.



**Figure 6-4. Set Browser Options Window**



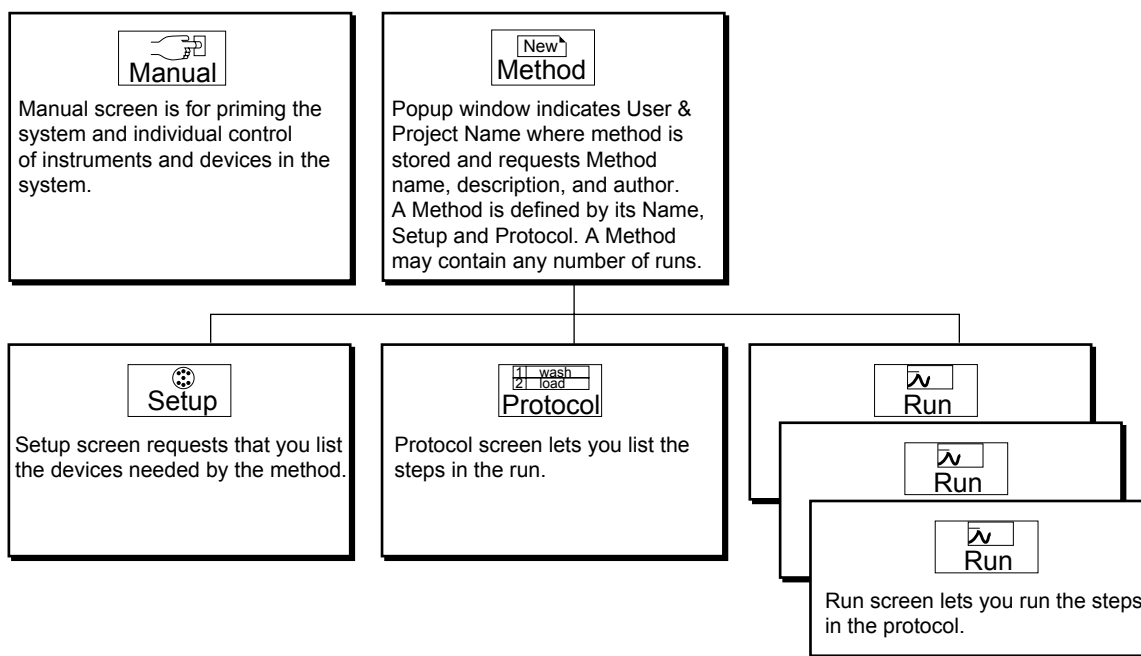


## 7.0 MODES OF OPERATION

There are four primary modes of operations, each represented by a screen display.

- Manual Control mode: To control the devices and instruments connected to the Workstation.
- Setup mode: Allows you define what instruments and devices will be used for any particular chromatography protocol, and if desired, to “alias” or name them for a specific task. For example, a SVT3-2 valve may be aliased as a “Fraction collector diverter valve.”
- Protocol mode: To define each of the steps to be run during a Method. Most steps are programmed using a dialog box to enter all of the parameter values for that step. Note that the number of the step being programmed is displayed in the upper left corner of the dialog box.
- Run mode: To run a method or view a run in progress.

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



**Figure 7-1. Relationships between Modes of Operation**

## 7.1 MANUAL CONTROL SCREEN

The Manual mode screen lets you control different devices and instruments on an individual basis.

**Note:** If an instrument is connected to the BioLogic Duo-Flow system on the instrument bus but does not appear in the Manual screen, check that its power switch is On and that the connection is secure.

BioLogic Duo-Flow - <user name> - <project name> - <method name>

File View Utilities Options Window Help

Method Method Run Browser Report Manual Setup Protocol Run Notes PostRun Log ON

<b>Gradient pump: F-10</b> Flow rate: 1.00 ml/min Inlet A: 100 % Inlet B: 0 % High limit: 400 psi Low limit: 20 psi Set START STOP	<b>Fraction collector</b> Mode: <input checked="" type="radio"/> System <input type="radio"/> Local Rack: Rack 1 (128 tubes) Start Tube: 1 End Tube: 128 Fraction size: 1.00 ml Tube number: 1 Volume left: Advance START STOP	<b>UV Detector</b> Zero Baseline ON OFF <b>Chart Recorder</b> Conductivity range(mS/cm): 500 UV range (AU): 0.2 Event mark ON OFF <b>Signal Import Module</b> SIM 1 and SIM 2 connected
---	---	--

<b>SVT3-2 at port 1</b> 1 <input checked="" type="radio"/> 2 <input type="radio"/>	<b>SV5-4 at port 2</b> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 4 <input type="radio"/> 3 <input type="radio"/>	<b>AVR7-3 at port 4</b> I <input type="radio"/> L <input checked="" type="radio"/> P <input type="radio"/>	<b>AVR9-8 at port 5</b> I <input checked="" type="radio"/> 8 <input type="radio"/> 2 <input type="radio"/> 7 <input type="radio"/> 3 <input type="radio"/> 6 <input type="radio"/> 4 <input type="radio"/> 5 <input type="radio"/>
---	---	--	---

Gradient Pump: F-10	UV	Conductivity	SIM1/SIG	SIM2/pH
0.00 ml/min	0 %B	35 psi	0.548 Volt	7.00 pH

Figure 7-2. Manual Mode Screen, showing a Model 2128 Fraction Collector and Four Valves


From the Manual mode screen you can control the following:

- **Gradient Pump:** This control panel allows you to control the composition of the eluant being pumped, set the flow rate and pressure limits, and start/stop the pump. (User-defined pressure limit settings is discussed in the section below.) If the pressure limit is exceeded, the pumps stop. **Note:** Changing pressure limits requires pressing either the **Start** or **Set** buttons. When the pump is running, changing any parameters requires pressing the **Set** button.
- **Fraction Collector:** The system displays the “Tube number,” which is the number of the tube currently under the drop-head (assuming the run started at tube 1), and the “Volume left,” which is the volume remaining to be delivered. There are also toggle buttons for **Start** and **Stop** and a button for **Advance**. Note that the Tube number is a display field only.

There are two modes of operation: **System**, in which the full functionality of the unit is available from the BioLogic Duo-Flow system software, and **Local**, in which control of the fraction collector is from its front panel.

**Note:** Fraction collection is always by **Volume**. Collection by Drop or Time is not supported by the BioLogic Duo-Flow software.

- **UV Detector:** Allows you to turn the UV Lamp on and off and to zero the baseline reading.
- **Chart Recorder:** If you are using the UV and Conductivity Detectors supplied with the system, you can set their chart recorder full scale ranges, start/stop the recorder, and send an event mark. The full scale ranges set here do not apply to the chromatograms displayed on the computer screen.
- **Signal Import Module (SIM 1 and 2):** This panel indicates the presence of up to two Signal Import Modules (SIM-HR). A SIM-HR is an analog-to-digital device. The signal from an analog instrument connected to the SIM is digitized so that the instrument can be monitored from the BioLogic Duo-Flow system. Instruments that may be connected this way include any detection source, such as a variable wavelength UV detector, a pH meter, a refractive index detector, or a fluorescence detector. For more information, refer to Chapter 2.4.1 and to the separate instruction sheet for the SIM-HR module.
- **Valves:** Allows you to control any of the BioLogic Duo-Flow solenoid or automated valves.

In addition, you can display the chromatogram window. From the System Toolbar, press the  button to display the Manual Control screen's electronic strip chart recorder. It will be placed over the valve control panels. Use the scroll bars or the **Chromatogram Settings** selection under the **Options** drop-down menu to change the x- and y-axes settings.

### 7.1.1 User Pressure Limit Settings

The Duo-Flow system is designed for pressures to 3500 psi. If you will be using a column designed for lower pressures, you can set a lower pressure limit for the system. If the pressure limit is exceeded, the pumps stop. **Note:** Changing pressure limits requires pressing either the **Start** or **Set** buttons.

## 7.2 SETUP SCREEN

The Setup screen is used to define what instruments/devices will be used by the Protocol for a particular Method. Each Method is thus accompanied by its own Setup. The information that you provide in Setup is necessary to properly operate the system. For example, if you plan to use a Model 2128 Fraction Collector, then you must select this particular collector in the Setup screen.

If you routinely use the same Setup, then it is recommended to select **Save setup as default** from the File drop-down menu. This setup is then loaded each time you create a New Method, thus eliminating the need to enter a new Setup each time.

Devices are chosen from the left hand “Available Devices” box. A dialog box will appear allowing you to define or alias the function of the device in the Method. For example, an AVR7-3 may be selected and then defined as a Sample Inject Valve. Use the **Delete** button in the System Toolbar to remove a device from the Devices in Setup window. In order to **run** your Method, these devices must be connected to the rear of the Workstation and they must be powered on.

**Note:** Devices do not need to be connected to the system in order to include them in the Setup for a method. However, the Run will not start unless these devices are connected; an error message will appear on the Run screen.

Available Devices		Devices in setup	
Aux Load Pump	Fraction Collector	UV Detector	
UV Detector	Conductivity Monitor	Conductivity Monitor	
SIM1 pH	SIM1 Signal		
SIM2 pH	SIM2 Signal		
SVT3-2 Valve	SV5-4 Valve		
AVR7-3 Valve	AVR9-8 Valve		

Gradient Pump: F-10	
Inlet A:	Buffer A
Inlet B:	Buffer B

Gradient Pump: F-10	UV	Conductivity	SIM1/SIG ▼	SIM1/pH ▼
0.00ml/min	0 %B	35 psi	0.00229 AU	1.234 mS/cm
			0.548 Volt	7.00 pH

Figure 7-3. Setup Screen

**Notes on using Valve Functioning and Aliasing:**

One of the most powerful features of the BioLogic Duo-Flow Software is the ability to define the specific purpose of the solenoid and automated valves and to “alias” or name each valve position. This greatly simplifies programming individual steps in the Protocol screen.

For example;

- The most common use of an AVR7-3 valve is as a Sample Inject Valve. Once an AVR7-3 valve has been assigned to this role, the software controls all of its valve changes based on the programmed sample injection step.
- The most common use of a SVT3-2 valve will be as a Fraction Collector Diverter valve with a Model 2110 or generic collector. Once an SVT3-2 valve has been assigned to this role, the software controls eluant diversion based on the programmed fraction collection scheme.

In summary, you can identify the following as applying to all valves:

- **Valve Name/Function.** What is the valve to be used for? Select from the drop-down list box or input a unique function that you will recognize when the particular valve is selected in the Protocol screen.
- **Connector.** Which connector at the rear of the workstation is the valve connected to?
- **Position Names.** These fields let you name (“Alias”) each valve position.

The following devices are listed in the Setup screen:

- **Auxiliary Load Pump:** This button should be selected if you are using an Auxiliary Load Pump, such as the Model EP-1 Econo Pump. See Section 4, Advanced System Applications, for ways to incorporate an auxiliary load pump into a method.
- **Fraction Collector:** Select this button if you are using a fraction collector with the system. A dialog box appears, allowing you to select which collector is to be used. If you use threshold detection to initiate fraction collection, choose the detector source in this screen.  
**Note:** To enable the use of collection windows or threshold collection for a Model 2110 or generic fraction collector, it is necessary to assign an SVT3-2 valve as the fraction collector diverter valve.
- **UV Detector:** Typically, the UV Detector is always listed in the Devices in Setup panel, as it is standard with the BioLogic Duo-Flow system.
- **Conductivity Monitor:** Typically, the Conductivity Monitor is always listed in the Devices in Setup panel, as it is standard with the BioLogic Duo-Flow system.
- **Signal Import Modules (SIMs):** The analog devices which may be used with the Signal Import Module-HR include any detection source, such as a variable wavelength UV detector, a pH meter, a refractive index detector, or a fluorescence detector.

Pressing the **SIM pH** button adds the device to the Devices in Setup list. Pressing the **SIM Signal** button displays the **Edit Signal Import Module** dialog box. Set the **Units** and the minimum and maximum for the **Units Range** and **Device Output Range (volts)**. Select the device number on the SIM-HR to match the number displayed in the dialog box. Refer to the analog device's documentation for information on output voltages.

- **Valves (SVT3-2, SV5-4, AVR7-3, and AVR9-8):** The BioLogic Duo-Flow system's automatic valves can be configured in a number of different ways depending on their function in a method. Table 7-1 discusses how each of the valves can be set up. See Section 4, Advanced System Applications, for ways to use and alias valves.
- **Gradient Pump:** Inlets A and B may be aliased by overwriting “Buffer A” and “Buffer B” with the description of the buffer.

**Table 7-1.  
Valve Setup Information**

Valve Type	Valve Name/ Function	Position Names	Notes
<b>SVT3-2</b> Low pressure solenoid valve	Fraction Collector Diverter	1 Waste 2 Collect	Eliminates the need for a “Change Valve” step in the Protocol screen, since its function as a fraction collection diverter is determined by the actual fraction collection parameters chosen.
	Inlet A	Aliased by user	When used before the inlet to Pump A or B, the valve enables buffer selection. The buffer name/alias specified for each position will appear in the Protocol screen’s “Isocratic Flow”, “Linear Gradient”, and “Change Valve” dialog box. Refer to Chapter 8, Sample Loading, for examples.
	Inlet B	Aliased by user	
	Aux Pump Inlet	Aliased by user	Used for auxiliary pump load selection to select one of two solutions. Refer to Chapter 8, Sample Loading, for examples.
	User-assigned name	Aliased by user	When used for a purpose other than that described above. The name/alias specified for each position will appear in the Protocol screen’s “Change Valve” dialog box.
<b>SV5-4</b> Low pressure solenoid valve	Inlet A	Aliased by user	When used before the inlet to Pump A or B, the valve provides preparative sample loading or buffer selection. The buffer or sample name/alias specified for each position will appear in the Protocol screen’s “Isocratic Flow”, “Linear Gradient”, and “Change Valve” dialog box. Refer to Chapter 8, Sample Loading, for examples.
	Inlet B	Aliased by user	
	Aux Pump Inlet	Aliased by user	Used for Auxiliary Pump load selection to select one of four solutions. Refer to Chapter 8, Sample Loading, for examples.
	User-assigned name	Aliased by user	When used for a purpose other than that described above. The name/alias specified for each position will appear in the Protocol screen’s “Change Valve” dialog box.
<b>AVR7-3</b> High pressure valve	Sample Inject	1 Load Sample 2 Inject Sample 3 Purge	For automatically loading a sample.

**Table 7-1. (continued)**  
**Valve Setup Information**

Valve Type	Valve Functions	Valve Positions	Notes
AVR7-3 (cont'd)	User-assigned name	Aliased by user	When used for a purpose other than that described above. The name/alias specified for each position will appear in the Protocol screen's "Change Valve" dialog box. Refer to Section 4, Advanced System Applications, Chapters 8 through 10.
<b>AVR9-8</b> High pressure valve	Aux Pump Inlet	Aliased by user	Used for Auxiliary Pump load selection to select from up to eight samples, buffers, or rinse solution.
	Inlet A	Aliased by user	When used before the inlet to Pump A or B, the valve provides preparative sample loading or buffer selection. The buffer or sample name/alias specified for each position will appear in the Protocol screen's "Isocratic Flow", "Linear Gradient", and "Change Valve" dialog box. Refer to Section 4, Advanced System Applications, Chapters 8 and 9.
	Inlet B	Aliased by user	
	Fraction Collector	1 Waste 2 Collect 3-8 Aliased by user	Useful for collecting large volume samples. Collects up to 8 samples. The name/alias specified for each position will appear in the Protocol screen's "Change Valve" dialog box.
	User-assigned name	Aliased by user	When used for purposes other than those described above. The name/alias specified for each position will appear in the Protocol screen's "Change Valve" dialog box. Refer to Section 4, Advanced System Applications, Chapters 8 and 9.



### 7.3 PROTOCOL SCREEN

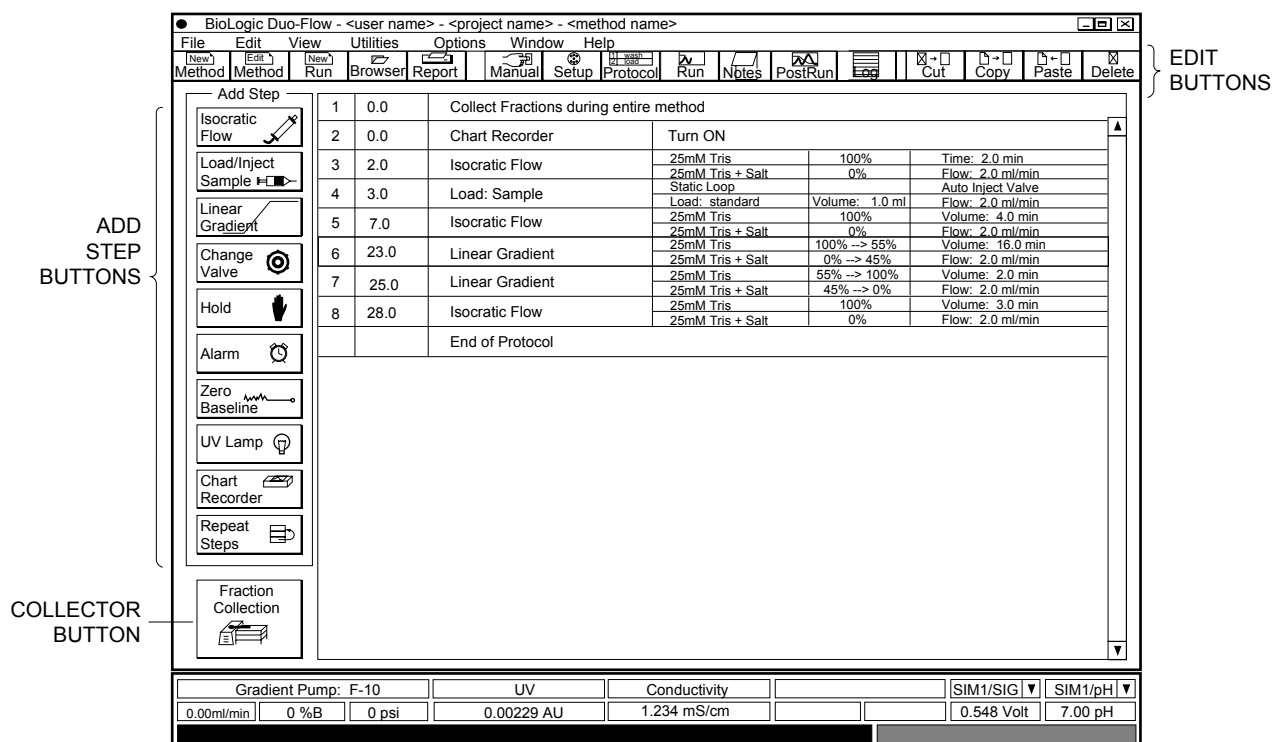
The Protocol screen provides a means to program step-by-step instructions to the system regarding what to do and when to do it.

**Note:** The programming base is either Volume (ml) or Time (min). The default programming base is set by selecting **Edit User Preferences** from the Options drop-down menu.

The Protocol screen contains the following types of buttons, which are described in detail in this section.

- **Add Step buttons:** These buttons are used to define the steps in the protocol. They are located in a vertical box on the left side of the screen. To insert a step, select the step *below* where the step is to be added, and then press the appropriate button to begin defining that step.
- **Fraction Collector button:** This button is used to define the fraction collection scheme. It is located below the Add Step buttons.
- **Edit buttons:** These buttons, located in the System Toolbar, are used to cut, copy, paste, and delete steps from the method. To cut, copy, or delete a step, select that step and then press the appropriate button. To paste, select the step below where the step is to be pasted and then paste.
- **File Buttons:** Use the New Method, Edit Method, and Browser System Toolbar buttons to create a new method or to open an existing Method.

The system checks the Setup information before allowing you to add steps or run the Method. This ensures that each device you need is present and available for the required function.

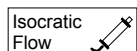


**Figure 7-4. Protocol Screen**

The following tables describe each button in the “Add Step” column of the Protocol screen.

**Table 7-2.**  
**Isocratic Flow Button**

Isocratic Flow		Buffers:		% Composition	Volume (ml)	OK
Start Volume (ml)	0.00	Buffer A	▼	A 100	1.0	
		Buffer B	▼	B	Flow (ml/min)	Cancel
					2.0	

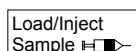


To deliver a buffer under isocratic conditions. The composition may be chosen by altering either Inlet A or Inlet B percent values which increase or decrease in tandem. Use the spin arrows or input values from the keyboard when this field is highlighted.

- **Start Time/Volume:** Calculates the elapsed time/volume from all previous steps. This is not user editable.
- **Buffers:** Displays all available buffers for separation. Buffer A and Buffer B can be aliased to actual buffer names by editing the Gradient Pump fields in the Setup screen. If pump inlet valves are assigned in the Setup screen, all additional buffers will be displayed in the drop-down boxes.
- **Composition:** This allows you to choose the buffer composition (e.g., 75% A and 25% B).
- **Volume/Time:** Allows you to choose the duration of the step.
- **Flow rate (ml/min):** Allows you to choose the flow rate of the step.
- **OK:** Adds the step to the protocol. This is the same as pressing the Enter key on the keyboard.
- **Cancel:** Does *not* add the step to the protocol. This is the same as pressing the Esc key on the keyboard.

**Table 7-3.**  
**Load/Inject Sample Button**

4 Load/Inject Sample		<input checked="" type="radio"/> Static Loop <input type="checkbox"/> Fill before Inject <input type="checkbox"/> Rinse after Inject <input type="radio"/> Dynamic Loop <input type="checkbox"/> Fill before Inject <input type="radio"/> Direct Inject		OK												
Start Volume (ml) 2.0				Cancel												
Inject Sample Auto Inject Valve will move to INJECT at Start of step and return to LOAD at end of step.		<table border="1"> <thead> <tr> <th>Injection Buffers:</th> <th>% Composition</th> <th>Volume (ml)</th> <th>Flow (ml/min):</th> </tr> </thead> <tbody> <tr> <td>Buffer A ▼</td> <td>A 100</td> <td>1.0</td> <td>2.00</td> </tr> <tr> <td>Buffer B ▼</td> <td>B 0</td> <td></td> <td></td> </tr> </tbody> </table>			Injection Buffers:	% Composition	Volume (ml)	Flow (ml/min):	Buffer A ▼	A 100	1.0	2.00	Buffer B ▼	B 0		
Injection Buffers:	% Composition	Volume (ml)	Flow (ml/min):													
Buffer A ▼	A 100	1.0	2.00													
Buffer B ▼	B 0															

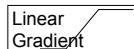


To program the sample injection. The Load Sample dialog box allows you to specify how the sample is to be introduced onto the column, as well as the volume of sample to be loaded. (If programming in time mode, the system calculates the time required for this step.)

- **Start Time/Volume:** Calculates the elapsed time/volume from all previous steps. This is not user editable.
- **Static Loop:** One of three options for sample loading. A static loop is a standard fixed volume sample loop. By adding an Auxiliary (AUX) pump and an auxiliary pump valve (such as the SVT3-2, SV5-4, or AVR9-8), the system can fill the sample into the static loop prior to injecting it into the column, and it can rinse the sample loop after the sample has been injected. **Note:** An isocratic step of sufficient length must precede a "fill before inject" step to allow sufficient time to fill the loop. Refer to Section 4, Advanced System Applications, for examples.
- **Dynamic Loop:** This is a second sample loading option which uses Bio-Rad's DynaLoop or other sliding-piston sample loop. Both partial loop and full loop injections can be programmed. By adding an Auxiliary (AUX) pump and an auxiliary pump valve (such as the SVT3-2, SV5-4, or AVR9-8), the dynamic loop can be filled prior to injection onto the column. The Rinse function is not available. The dynamic loop can also be filled manually through Inject valve port #2. Refer to Section 4, Advanced System Applications, for examples.
- **Direct Inject:** Allows direct injection of sample through either the Gradient Pump or Auxiliary Load Pump. Pre-pump valves (such as the SVT3-2, SV5-4, or AVR9-8) automate direct injection. Use of an auxiliary pump (such as the Bio-Rad Model EP-1 Econo Pump) allows direct injection of sample onto a low pressure column ( $\leq 15$ psi). Use of an Auxiliary (AUX) pump and an auxiliary pump valve allows multiple direct injections onto a low pressure column. Refer to Section 4, Advanced System Applications, for examples.
- **Inject Sample:** Explains the action of the inject valve. The AVR7-3 auto inject valve will move to the Inject position at the start of the step and return to the Load position at the end of the step.
- **Injection Buffers and % Composition:** These fields allow you to choose the buffer type and composition which will push the sample through the sample loop.
- **Volume:** Allows you to specify the volume of the sample to be injected. The smallest volume allowed is 0.1 ml; the largest volume allowed is 9999 ml (for larger volumes, use another Load/Inject Sample step).
- **Flow Rate:** Allows you to specify the flow rate for the sample injection step.
- **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel:** Does **not** add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.

**Table 7-4.**  
**Linear Gradient Button**

Linear Gradient		Buffers:	% Composition	Initial	Final	Volume (ml)	Flow (ml/min)	OK
Start Volume (ml)	2.0	Buffer A		A	0	100	1.0	Cancel
		Buffer B		B	100	0	1.00	

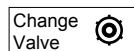


To deliver buffer gradients. The Linear Gradient dialog box sets the initial and final composition of the buffers and the period over which the change in composition is to occur. There is no limit to the number of gradient steps in a protocol.

- **Start Time/Volume:** Calculates the elapsed time/volume from all previous steps. This is not user editable.
- **Buffers:** Allows you to choose two buffers to make a binary gradient. Drop-down menus display all buffers that are set up using Gradient Pump Inlet valves.
- **% Composition:** Allows you to choose initial and final values that will form a linear binary gradient. Notice that changing one value in the “Initial” column affects the other value in that column. The “Final” column behaves identically.
- **Volume:** Allows you to choose the duration of this step.
- **Flow:** Allows you to choose the flow rate of this step.
- **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel:** Does *not* add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.

**Table 7-5.**  
**Change Valve Button**

Change Valve		Valve Name:	Valve Position:	OK
Start Volume (ml)	0.00	Sample Inject	Load Sample	Cancel



To select any valve and change its position.

- **Start Time/Volume:** Calculates the elapsed volume from all previous steps. This is not user editable.
- **Valve name and Valve position:** Allows you to choose a valve and to make a change in valve position. Note that certain valve functions defined in the Setup screen (such as Fraction Collector Diverter, Aux Pump Inlet, Sample Inject, Inlet A, Inlet B) are “tied” to other Protocol steps, so valve position changes will be made automatically. An example would be an AVR7-3 valve defined as a Sample Inject valve in the Setup screen, which is then tied to the Load/Inject Sample step in the Protocol screen.

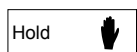
However, valves assigned a “User-defined name” during Setup require the “Change Valve” step at the desired point in the Protocol.

**Table 7-6.**  
**Hold Button**

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Hold Until Event		Event:	<input type="checkbox"/> Sound Alarm	OK
Start Volume (ml)	0.0	Hold until Keypress		Cancel

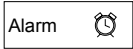
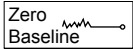
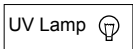
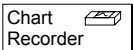
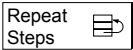
---




Inserts a Hold step into the protocol. During a Hold, the method's current Gradient Pump composition (%B) remains constant. The method time (or volume) advances. When the run resumes, the current fraction collection condition is maintained. The hold condition is maintained until a specified activity occurs (e.g., "Hold until Start of Inject" or "Hold until Keypress"). For example, if the hold starts at time 2 (minutes), then the method resumes at time 2, no matter how long the Hold was in effect.

- **Start Time/Volume:** Calculates the elapsed time/volume from all previous steps. This is not user editable.
  - **Event:** Choose one of three events to discontinue the hold:  
**Hold until Keypress:** Press the **F2** key on the keyboard to end the programmed hold.  
**Hold until Start of Inject:** The run will continue when the manually-controlled device (which must be connected to the Workstation AUX connector at pin 1, Inject) is moved to its desired position.  
**Hold until End of Inject:** The run will continue when the manually-controlled device (which must be connected to the Workstation AUX connector at pin 1, Inject) is returned to its original position.
  - **Sound Alarm:** When this box is checked, an alarm will sound at the beginning of the step to remind you that the system requires an action on your part that will then allow the rest of the method to advance.
  - **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
  - **Cancel:** Does *not* add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
-

**Table 7-7.**  
**Miscellaneous Buttons**

Button	Description
	To sound a 10 second audible alarm at the programmed time. The progress of the run will not be affected.
	To zero the UV baseline at the programmed time.
	At system start-up, the default condition is Lamp ON. If the lamp is OFF, it takes about 20 minutes for the lamp to warm up prior to use. This feature is particularly useful for overnight runs. Turning off the lamp after the run is complete will extend the lamp life.
	To turn on/off the Chart Recorder at the programmed time. Inserts a step that tells the Chart Recorder to start/stop the paper feed and lower/lift the pen.
	To repeat the highlighted step(s) a specified number of times. To highlight more than one step, hold down the Ctrl key while selecting steps with the mouse.

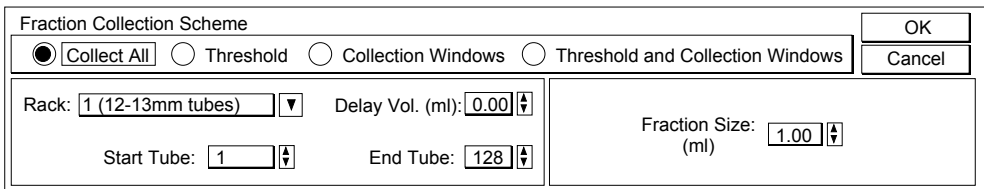
**Table 7-8.**  
**Fraction Collection Button**

Button	Description
	To set up fraction collection. Fraction collection schemes are discussed below.

### IMPORTANT NOTE:

The BioLogic Duo-Flow system is designed to control the Model 2128 Fraction Collector, which is the only collector that can provide a choice of racks and the ability to overlay fractions from consecutive runs.

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



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

- **Rack:** This applies only to the Model 2128 Fraction Collector. The following racks may be specified:
  - 1 128 tubes; 12-13 mm tube diameter
  - 2 78 tubes; 16-18 mm tube diameter
  - 3 Microplates, 3 total for 288 wells (96 wells/plate, arranged 8x12)
  - 4 Microtubes (128 capless microtubes)
  - 5 Prep-Adapter (10 bottles of any size)
- **Start Tube** and **End Tube:** This applies only to the Model 2128 Fraction Collector. Identify the first and last tubes to receive fractions.
- **Delay Vol. (ml):** The Delay function precisely synchronizes fraction collection with the UV signal on the chart recorder. It is defined as the tubing volume between the UV detector and the fraction collector drop-head. If you are using the recommended 1/16" (1.6 mm) OD, 0.020" (0.51 mm) ID PEEK tubing, 1 cm of tubing has a volume of 2 µl. The volume of the supplied back-pressure regulator is 146 µl.
- **Fraction Size:** Enter the fraction size as volume (ml).

**Table 7-8. (continued)**  
**Fraction Collection Button**

---

**Description**

---

Fraction Collection Scheme				OK
<input type="radio"/> Collect All <input checked="" type="radio"/> Threshold <input type="radio"/> Collection Windows <input type="radio"/> Threshold and Collection Windows				Cancel
Rack: 1 (12-13mm tubes) ▼	Delay Vol. (ml): 0.00 ▲▼	Fraction Size: 1.00 ▲▼	Non-peak Parameters:	
Start Tube: 1 ▲▼	End Tube: 128 ▲▼	Threshold: 0.0100 ▲▼	Destination	Fraction Size
			<input checked="" type="radio"/> Waste (ml) <input type="radio"/> Tubes	1.00 ▲▼

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

- **Rack, Start Tube, End Tube, Delay Vol. (ml), and Fraction Size:** See description for **Collect All**.
  - **Threshold:** Enter the global threshold value in absorbance units.
  - **Non-peak parameters Destination and Fraction Size:** Enter the non-peak parameters. If the buffer stream is diverted to Waste, there is no need to enter a fraction size. But if you want to collect non-peak material, you must enter a fraction size.
-



**Table 7-8. (continued)**  
**Fraction Collection Button**

---

**Description**

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**Collection Windows:** This method specifies collection during specified parts of the run (Time or Volume). Each collection window can have a unique fraction size (ml). **Note:** Collection windows programming should be done after all other steps have been written.

When using the Collection Windows functions, the Model 2128 will skip a tube between each window collection which is not linked (see below, Link Windows). For example, if you specify two windows for collection, where the first window ends collection at 20 minutes and the second window starts collection starting at 21 minutes, then the fraction collector will leave an empty tube for the period between minutes 20 and 21.

- **Rack, Start Tube, End Tube, Delay Vol. (ml), and Fraction Size:** See description for **Collect All**.
  - **Link Windows:** Select this to join windows, so that the end of one window is the beginning of another. It is particularly useful as an editing feature which, if selected, will automatically change the time. For example, it will change the end time of one window if the beginning time of the next collection window is altered.
  - **Start (ml or min), End (ml or min), and Fraction Size (ml):** Each window is defined by these parameters. Up to three windows can be displayed at any one time; use the scroll bar to the right of the window display to scroll through the list of window. Enter the fraction size as volume.
  - **Add Window and Delete Window:** The **Add Window** button inserts a window after the highlighted window; **Delete Window** deletes the highlighted window.
-

**Table 7-8. (continued)**  
**Fraction Collection Button**

---

**Description**

---

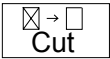

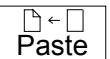

**Threshold and Collection Windows:** Each Collection Window can also contain a threshold value to further discriminate when eluant can be collected. For each collection window, non-peak effluent can be directed to waste or to the fraction collector.

When using the Collection Windows functions, the Model 2128 will skip a tube between each window collection which is not linked (see below, Link Windows). For example, if you specify two windows for collection, where the first window ends collection at 20 minutes and the second window starts collection starting at 21 minutes, then the fraction collector will leave an empty tube for the period between minutes 20 and 21.

- **Rack, Start Tube, End Tube, Delay Vol. (ml), and Fraction Size:** See description for **Collect All**.
  - **Link Windows:** Select this to join windows, so that the end of one window is the beginning of another. It is particularly useful as an editing feature which, if selected, will automatically change the time. For example, it will change the end time of one window if the beginning time of the next collection window is altered.
  - **Start (ml), End (ml), and Fraction Size (ml):** Each window is defined by these parameters. Up to three windows can be displayed at any one time; use the scroll bar to the right of the window display to scroll through the list of window. Enter the fraction size as volume (ml).
  - **Add Window and Delete Window:** The **Add Window** button inserts a window after the highlighted window; **Delete Window** deletes that window.
  - **Threshold:** Enter the threshold value for each collection window.
  - **Non-peak parameters Destination and Fraction Size:** Enter the non-peak parameters. When collecting non-peak material, you must enter a fraction size.
-

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

**Table 7-9.**  
**Protocol Screen's Editing Toolbar Buttons**

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

## 7.4 RUN SCREEN

The Run screen starts and displays the progress of the Method Run. All data associated with the run are automatically saved. An example of a run in progress is shown in Figure 7-5 below. Table 7-10 discusses the buttons which may be used to control the run. In addition, there are toolbar buttons that display additional screens associated with a completed run (**Post Run** and **Integ.**). These are discussed in the following sections.

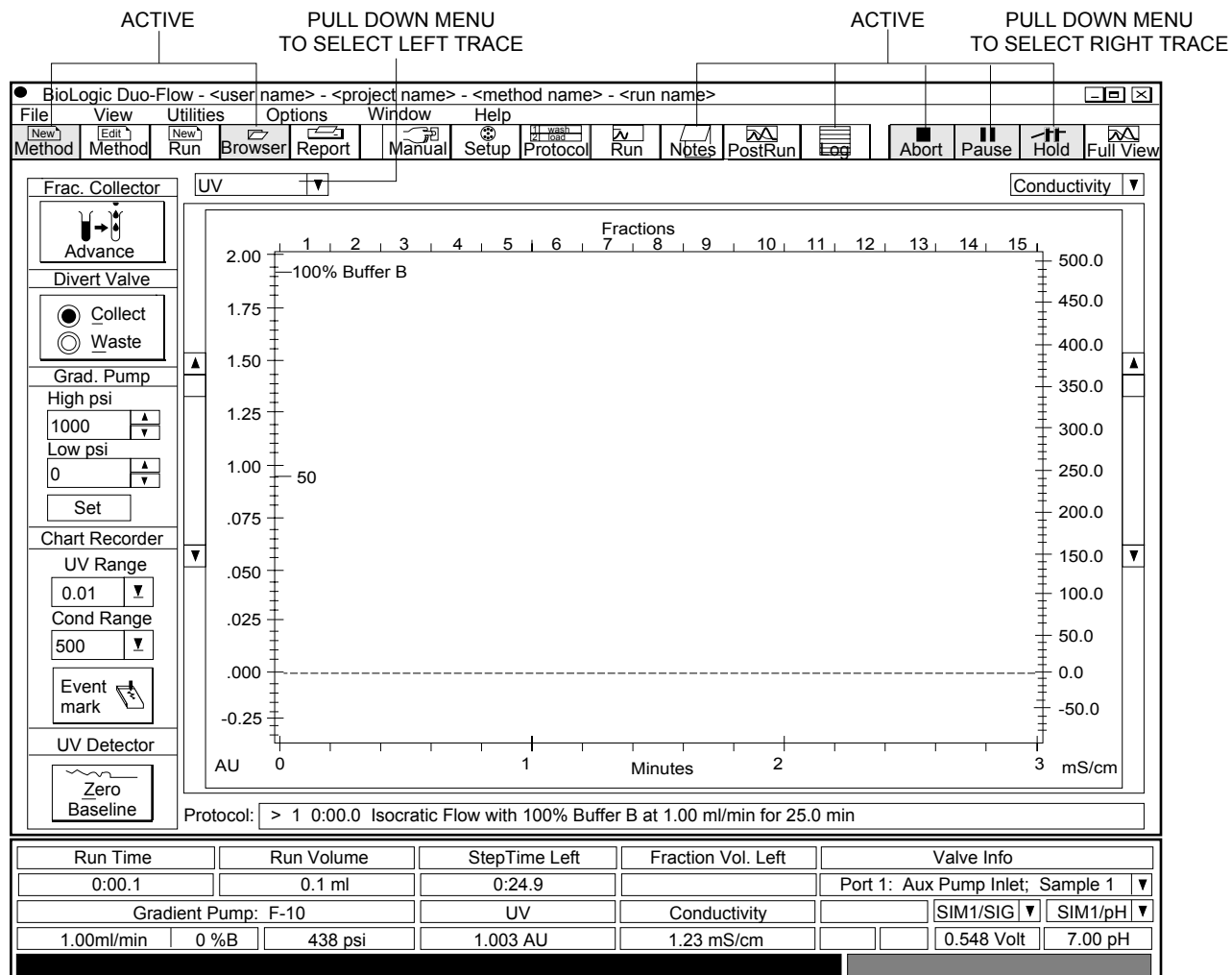


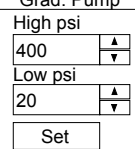
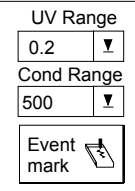
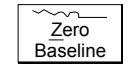
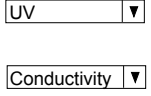





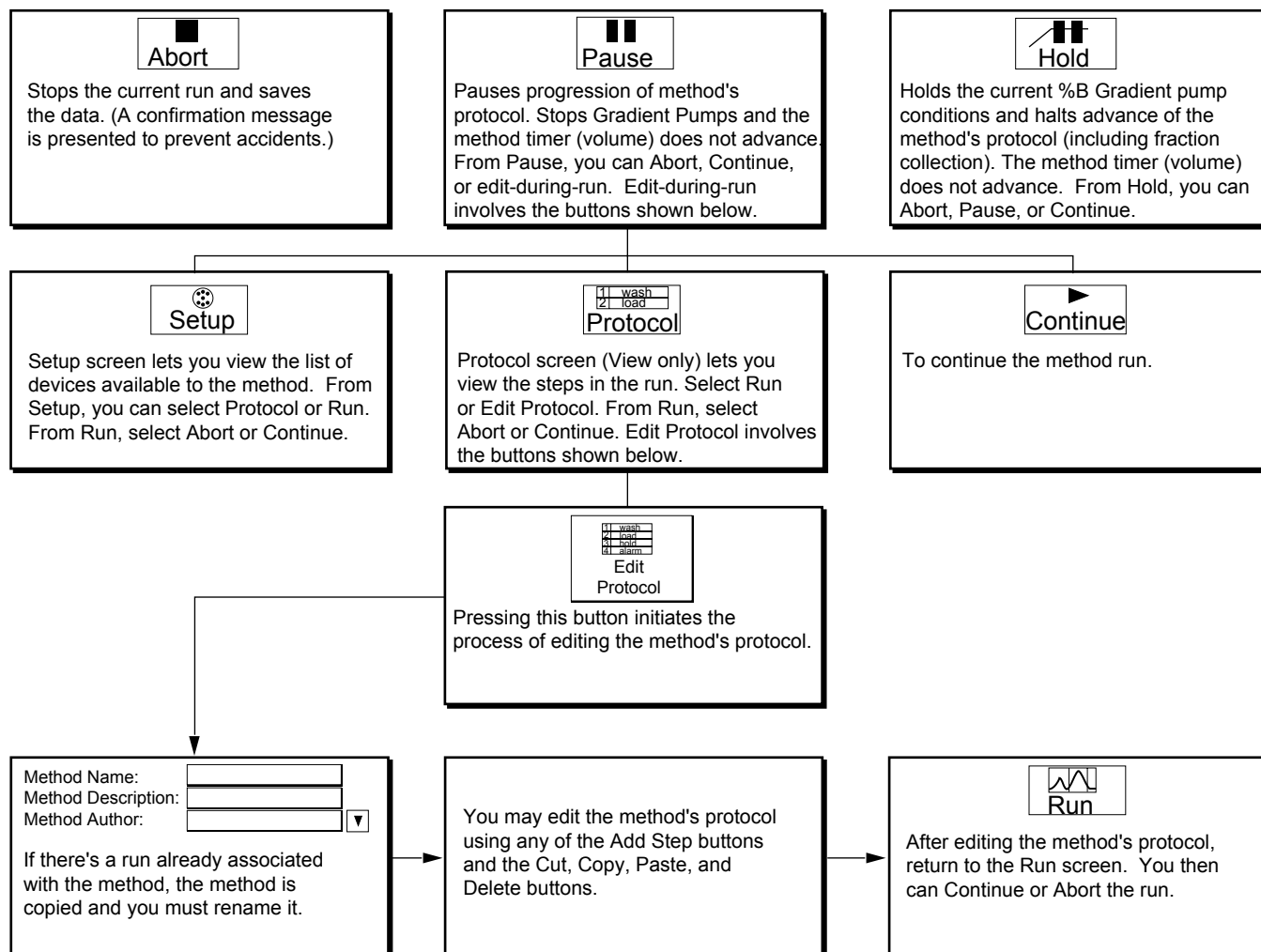
Figure 7-5. Run Screen showing a Run in Progress

**Table 7-10.**  
**Run Screen's Control Buttons**

Button	Description
	Advances the fraction collector to the next tube. Pressing this button does not modify the method. The event is recorded in the Run Log.
	Immediately changes the position of the diverter valve. Pressing this button does not modify the method. The event is recorded in the Run Log.
	To set the pressure limits of the Gradient Pump. This is not part of a protocol; it is a system setting.
	<p>To set the <b>UV Detector</b> range for the Chart Recorder output. The settings are: 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 AUFS.</p> <p>To set the <b>Conductivity</b> range on the Chart Recorder output. The settings are: 500, 200, 100, 50, 20, 10, 5 mS/cm.</p> <p>To set an <b>Event Mark</b> on the chart recorder UV trace output only. It will not record event marks on the screen.</p>
	Use the <b>Zero Baseline</b> button to reset the UV detector absorbance value to zero. <b>Note:</b> Use care when using this feature during a collection scheme which uses a <b>Threshold</b> value.
	These drop-down menus, located across the top of the chromatogram, are used to select which of the active data trace scales are scaled during a run. As each trace scale is selected, the scroll bars can be used to adjust the scale setting.
	To work offline while a run is in progress. (Refer to section 7.4.2, Working Offline.)
	Notes displays the Run Notebook screen, which allows you to enter notes regarding the run. Log displays the Run Log screen, which provides information about the run and is non-editable.
	To stop, pause, or hold a method in progress. (Refer to Figure 7-6.)

### 7.4.1 Pausing/Stopping a Method in Progress

The Run screen provides three buttons for pausing or stopping a method in progress. These buttons are discussed in the figure below.



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

### 7.4.2 Working Offline

The BioLogic Duo-Flow system software allows you to work offline while a run is in progress. The following actions can be performed:

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

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

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

During a run, the following toolbar buttons activate the offline window:

- **New Method** button
- **Browser** button

The online and offline windows are distinguished as follows:

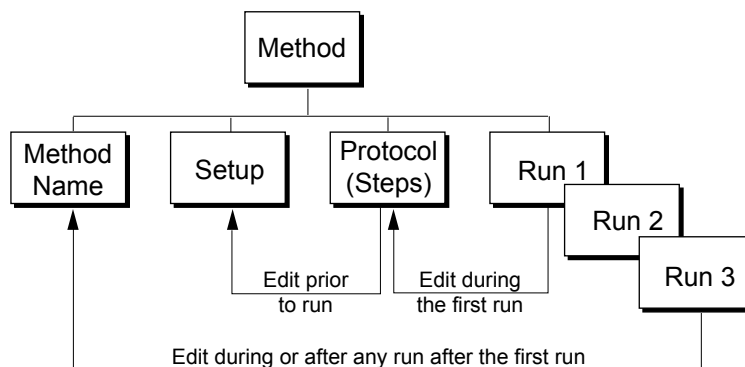
- Online: The BioLogic Duo-Flow icon in the upper left of the screen is green, and the title bar does not indicate online.
- Offline: The BioLogic Duo-Flow icon in the upper left of the screen is yellow, and the title bar indicates offline.

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

- Windows drop down menu: The online and offline windows are listed.
- Task bar: The Windows® 95 taskbar lists the BioLogic Duo-Flow windows.
- Alt-Tab: Simultaneously hold down these two keys to list the open windows.

### 7.4.3 Editing A Protocol While a Run is In Progress

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



**Figure 7-7. Editing during a Run**

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

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

Upon completion of the run, the database of Methods and Runs will show three runs associated with the method “Ion-exchange UNO Q1” and one run associated with the method “Ion-exchange UNO Q1 Rev. 1.”

- A fraction collection scheme cannot be added to a Protocol once a run has been started. In such cases, you should abort the run and select **Edit the Method**.
- A currently programmed fraction collection scheme may be edited during a run, but note that changing the fraction size of a “Collect All” scheme will first require selection of “Collection Windows.” This is because the editing process effectively turns the initial “Collect All” scheme into the “first” collection window.



● BioLogic Duo-Flow - <user name> - <project name> - <method name>

File Edit View Utilities Options Window Help

Method Method Run Browser Report Manual Setup Protocol Run Notes PostRun Log Cut Copy Paste Delete

Add Step:

1 0.0 Collect Fractions during entire method

2 0.0 Chart Recorder Turn ON

3 2.0 Isocratic Flow 25mM Tris 100% Time: 2.0 min

4 3.0 Load: Sample 25mM Tris + Salt 0% Flow: 2.0 ml/min

5 7.0 Isocratic Flow Load: standard Volume: 1.0 ml Auto Inject Valve

6 23.0 Linear Gradient 25mM Tris 100% --> 55% Flow: 2.0 ml/min

7 25.0 Linear Gradient 25mM Tris + Salt 0% --> 45% Flow: 2.0 ml/min

8 28.0 Isocratic Flow 25mM Tris 55% --> 100% Volume: 2.0 min

25mM Tris + Salt 45% --> 0% Flow: 2.0 ml/min

25mM Tris 100% Volume: 3.0 min

25mM Tris + Salt 0% Flow: 2.0 ml/min

End of Protocol

Gradient Pump: F-10 UV Conductivity SIM1/SIG SIM1/pH

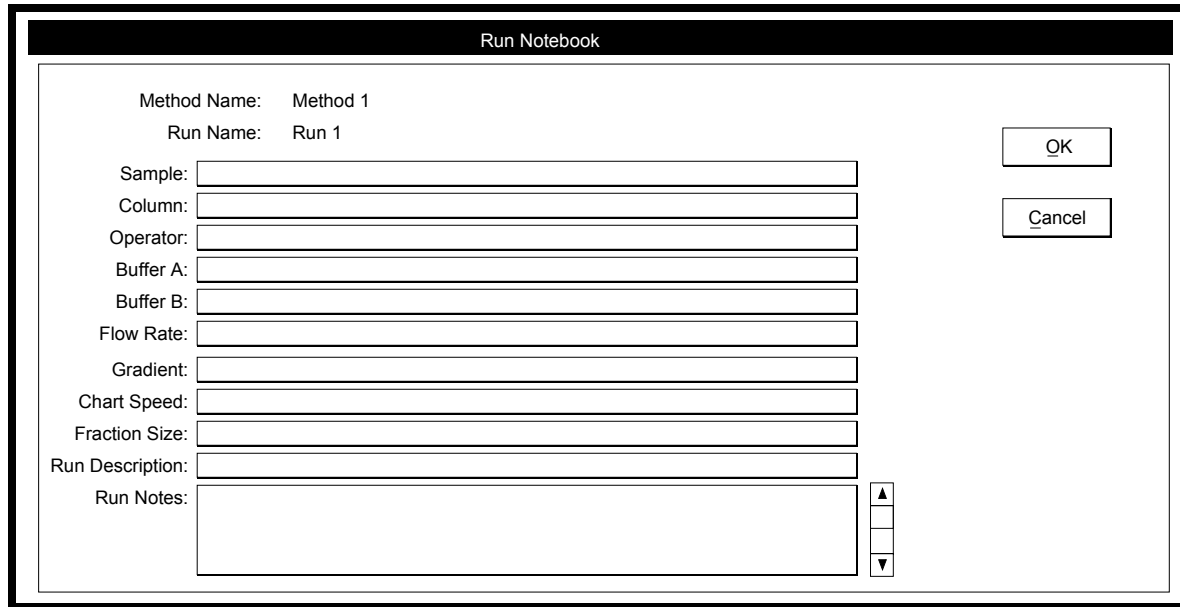
0.00ml/min 0 %B 35 psi 0.00229 AU 48 mS/cm 0.989 Volt 4.00 pH

Select item(s) for copy/cut/delete. Double-click on step to edit.

Figure 7-8. Protocol Screen during a Run

#### 7.4.4 Run Notebook Screen

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



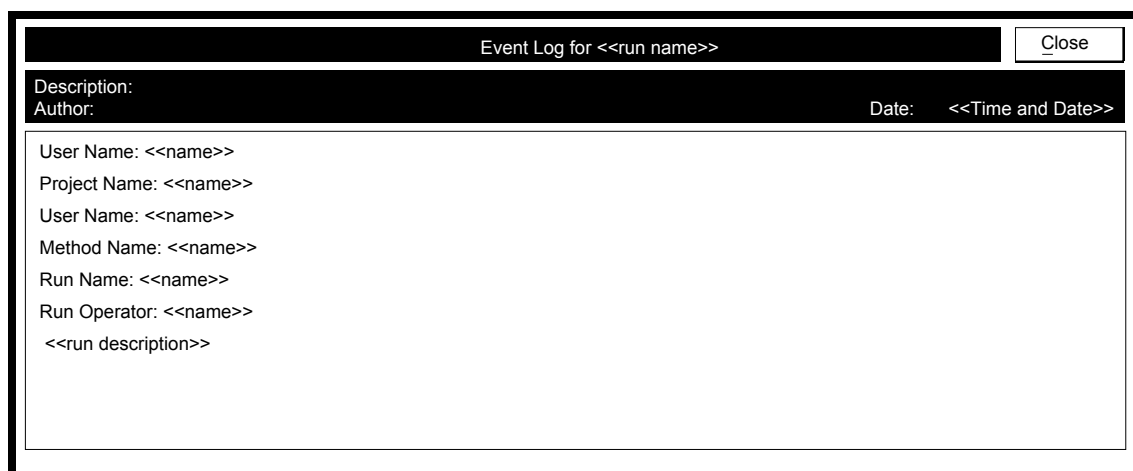
The Run Notebook screen is a dialog box with a title bar labeled "Run Notebook". It contains the following fields and controls:

- Method Name: Method 1
- Run Name: Run 1
- Sample: [Text Field]
- Column: [Text Field]
- Operator: [Text Field]
- Buffer A: [Text Field]
- Buffer B: [Text Field]
- Flow Rate: [Text Field]
- Gradient: [Text Field]
- Chart Speed: [Text Field]
- Fraction Size: [Text Field]
- Run Description: [Text Field]
- Run Notes: [Text Area]
- OK button
- Cancel button
- Up, Down, and Enter arrow buttons next to the Run Notes field.

Figure 7-9. Run Notebook Screen

#### 7.4.5 Run Log Screen

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



The Run Log screen is a dialog box with a title bar labeled "Event Log for <<run name>>". It contains the following fields and controls:

- Close button
- Description: [Text Field]
- Author: [Text Field]
- Date: <<Time and Date>> [Text Field]
- User Name: <<name>> [Text Field]
- Project Name: <<name>> [Text Field]
- User Name: <<name>> [Text Field]
- Method Name: <<name>> [Text Field]
- Run Name: <<name>> [Text Field]
- Run Operator: <<name>> [Text Field]
- <<run description>> [Text Field]

Figure 7-10. Run Log Screen

## 7.5 POST-RUN SCREEN

The Post-Run screen enables peak annotation (“tagging”) and data export. To enter the Post-Run screen, open a Run file using the **Browser** button on the button bar, or when a run is finished choose **PostRun** from the Toolbar buttons. Two chromatograms are viewed: the main chromatogram in the lower large window and the reference chromatogram in the upper right window.

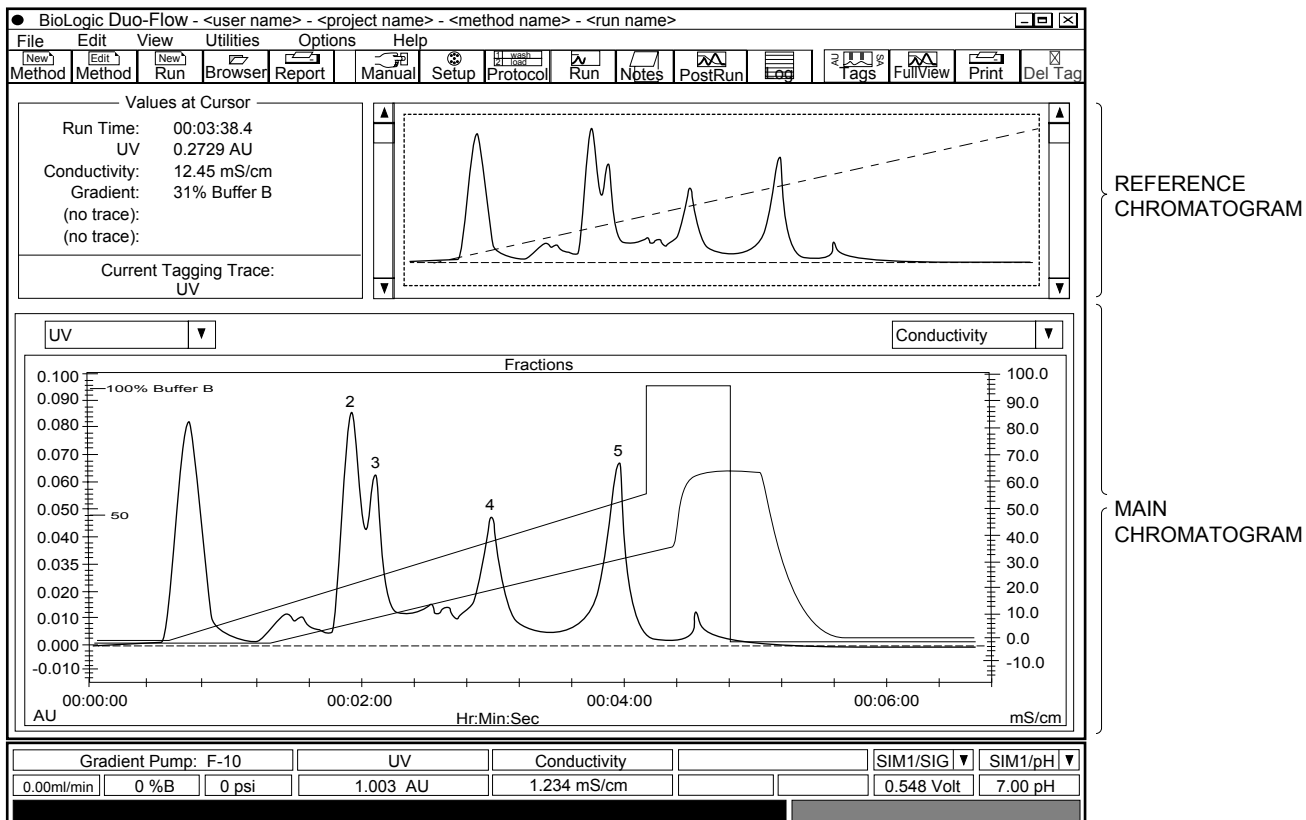


Figure 7-11. Post-Run Screen

### 7.5.1 Resizing

The main chromatogram shows the current “Zoom” region. To resize the image (or “zoom in” to a particular region of the chromatogram), either change the axes scale in the reference chromatogram, or use the “rubber band” controls on either chromatogram. To use the rubber band controls, click and hold the cursor within the chromatogram region and move the cursor so that the area of interest is enclosed. Release the cursor and the main chromatogram will be resized. You can then pan across the area by clicking the right mouse button and moving the mouse.

### 7.5.2 Chromatogram Information (Values at Cursor)

The top left corner of the PostRun screen contains information about the chromatogram. The position of the vertical bar within the main chromatogram dictates the displayed information. Run time, the absorbance (AU) and conductivity (mS/cm) values, gradient progression (%B), and the current tagging trace are displayed. To view the vertical bar, move the cursor inside the main chromatogram.

### 7.5.3 Annotating ("tagging") the Chromatogram

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

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

**Post Run Tags**

Trace	Trace Visibility	Tag Visibility	Active Trace
UV (AU)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="radio"/>
Cond. (mS/cm)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>
(%) Buffer B	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>
(no trace)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>
(no trace)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>

**Tag Style for active trace**

☒ Sequential Tag Number  
☐ User Tag Name  
☐ Trace Value (Y axis value)  
☐ Run Time (X axis value)

Ok Cancel

**Tags for UV trace**

Tag #	Tag name	Retention time (h:m:s)	Volume (ml)	Buffer B (%B)	UV (AU)	Cond. (mS/cm)	(no trace)	(no trace)	Tube #
1	Myoglobin	00:00:32	2.2	0	0.07992	1.496	N/A	N/A	0.07992
2	Conalbumin, peak 1	00:01:41	6.7	18	0.08617	7.999	N/A	N/A	0.08617
3	Conalbumin, peak 2	00:01:48	7.2	20	0.05921	9.025	N/A	N/A	0.05921
4	Ovalbumin	00:02:44	11.0	34	0.03799	16.472	N/A	N/A	0.03799
5	Soybean Trypsin Inhibitor	00:03:30	14.0	46	0.06844	22.308	N/A	N/A	22.308

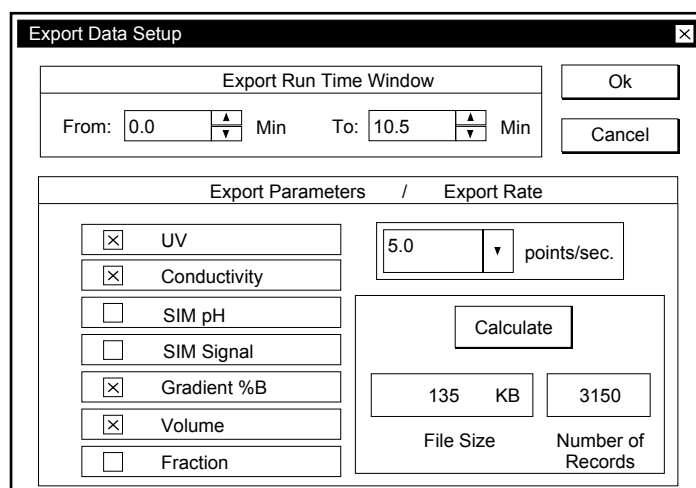
**Figure 7-12. Post-Run Tags**

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

### 7.5.4 Transferring Data into other Programs

To transfer data, go to the Post-Run screen of the desired run. Select **Export Data** from the File drop-down menu (See Figure 7-13, Export Data Setup screen). From this screen, choose the run time and the data trace(s) that will be exported. Although data is collected at a rate of five points per second, it can be exported at a user-defined rate. Data is exported in an ASCII.TXT file format. The file name is user-defined and can be saved anywhere on the hard drive. Note that the default file is called "Export" and is located in the following directory, "Biologic/data/export".

When importing the data into Excel, choose comma delimited.



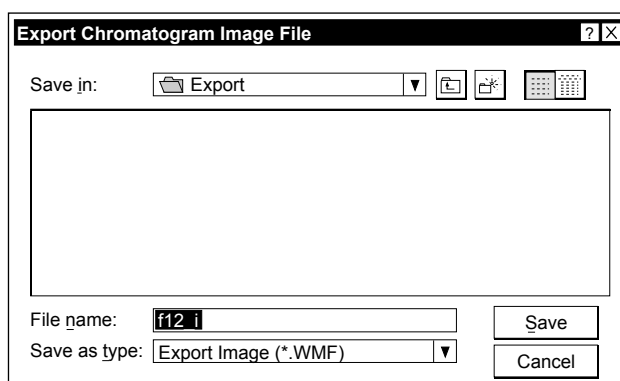
**Figure 7-13. Export Data Setup Screen**

### 7.5.5 Exporting Chromatogram Images

Chromatographic images can be exported into other applications, such as word processing programs. Before transferring an image, modify the chromatogram in the PostRun screen since changes can not be made to the file once it is transferred out of the BioLogic Duo-Flow program. The image in the main chromatogram will be the exported image.

Select Export Chromatogram Image from the File drop-down menu (see Figure 7-14 46). The image is exported as a Windows Meta File (\*.wmf) format. In general, Windows Meta File format is used for transfer into a Windows-based application.

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



**Figure 7-14. Exporting a Chromatographic Image**



## 8.0 SAMPLE LOADING

The BioLogic Duo-Flow system supports several methods for sample loading. Typically a sample is loaded through a static fixed volume loop, as shown in chapter 4. When large volume sample injections are required, the following options are available:

- sample loading through the Workstation Gradient Pump
- sample loading using the DynaLoop sliding-piston loop, available in both 25 and 90 ml volumes
- sample loading onto a low pressure column using the Econo Pump as an auxiliary (Aux) pump
- multiple sample loading using either an AVR9-8 valve or DynaLoop

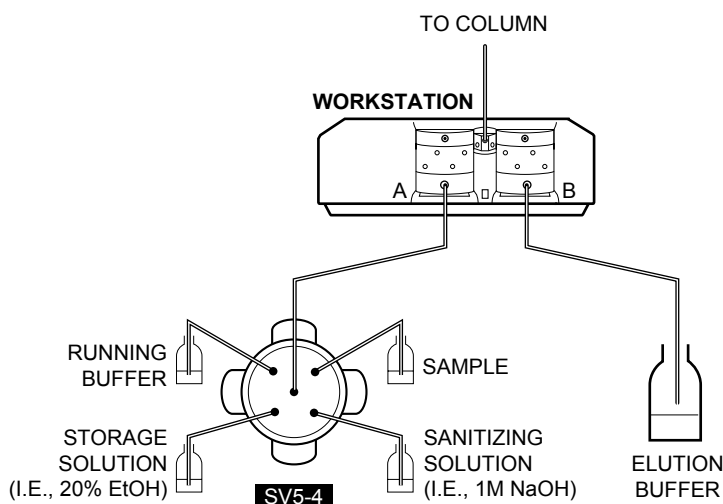
### 8.1 SAMPLE LOADING THROUGH THE WORKSTATION GRADIENT PUMP

Samples may be loaded directly through the Workstation Gradient Pump, as long as the sample is first filtered through a 0.45  $\mu\text{m}$  filter. Use a sanitizing solution to remove protein residues after the sample has been loaded; protein residues might otherwise reduce piston seal lifetime. Consult the On-Line Help for additional information.

#### 8.1.1 System Setup

When loading sample through the Workstation Gradient Pump, use an SVT3-2 or SV5-4 valve to facilitate sample loading and cleaning. Whichever valve is selected, it should be plumbed to Inlet A on the Gradient Pump. To prevent air from entering the system, unused ports should be closed with the 1/4-28 plugs which are supplied in the BioLogic Duo-Flow system fittings kit. In the Setup screen, where the Gradient Pump is aliased, Inlet A can be identified as "Sample Inject Valve."

In this example, one port of the SV5-4 valve is dedicated to the sample, another to running/equilibration buffer, a third to storage solution (such as 20% EtOH), and the fourth to a sanitizing solution (such as 1M NaOH). The elution buffer is plumbed directly into Gradient Pump B and is configured as Inlet B in the Setup screen.



**Figure 8-1. Sample Loading Through the Gradient Pump**

### 8.1.2 Writing the Protocol

The following steps should be programmed within the Method Protocol, as shown in the figure below:

1. Add an Isocratic Step to establish the running conditions.
2. At the Load/Inject Sample step, specify Direct Inject and choose Gradient Pump A as the inlet through which the sample will be delivered. Enter the volume of sample and the flow rate for the injection step. The step starting time or volume appears to the left of the options buttons.
3. Add an Isocratic Flow Step with Running buffer to ensure that all the sample has been moved out of the pre-pump inlet lines and onto the column. Three to five volumes of inlet tubing length is recommended. There is approximately 20 µl in 1 cm of 1/8" (3.2 mm) OD tubing. For example, for 10 cm of 1/8" (3.2 mm) OD tubing, program an isocratic flow step of 1.0 mL.  
(10 cm) (0.020 mL/cm) 5 = 1mL
4. Add a Change Valve step to move the AVR7-3 Sample Inject valve to the Purge position, taking the column off line, and add an Isocratic Flow step to run 10mL of buffer B through Gradient Pump B. This will ensure that the mixer has been removed of any protein residue. Add an Isocratic Flow step to run 10mL of Running buffer through the Gradient Pump A to establish Running Buffer conditions.
5. Add a Change Valve step to move the AVR7-3 Sample Inject valve back to the Load position to begin the run.
6. Continue writing the separation protocol.
7. For sanitization, 1.0M NaOH may be used. Be sure to wash with water to remove the base. Refer to Chapter 12, Maintenance, for information on how to store the BioLogic Duo-Flow system.

**Top Screenshot: Add Step Dialog**

Volume	Description	Parameters
1	Load/Inject Sample	<input type="radio"/> Static Loop <input type="checkbox"/> Fill before Inject <input type="checkbox"/> Rinse after Inject <input type="radio"/> Dynamic Loop <input type="checkbox"/> Fill before Inject <input checked="" type="radio"/> Direct Inject
Start Volume (ml)	0.0	<input type="radio"/> Pump A <input type="radio"/> Pump B Sample: [Dropdown] Volume (ml): 25.0 Flow (ml/min): 1.50

**Bottom Screenshot: Method Protocol Table**

Volume	Description	Parameters
1	0.0 Isocratic Flow	Running Buffer 100%, Elution Buffer 0%, Volume: 30.0 ml, Flow: 1.50 ml/min
2	30.0 Load/Inject Sample	Load: Sample, Gradient Pump A, Volume: 25.0 ml, Flow: 1.50 ml/min
3	55.0 Isocratic Flow	Running Buffer 100%, Elution Buffer 0%, Volume: 1.0 ml, Flow: 1.50 ml/min
4	56.0 Change Valve	Sample Inject Position: Purge
5	56.0 Isocratic Flow	Sample 0%, Elution Buffer 100%, Volume: 10.0 ml, Flow: 1.50 ml/min
6	66.0 Isocratic Flow	Running Buffer 100%, Elution Buffer 0%, Volume: 10.0 ml, Flow: 1.50 ml/min
7	76.0 End of Protocol	

**Figure 8-2. Writing a Protocol for Sample Injection Through the Gradient Pump**



## 8.2 SAMPLE LOADING USING THE DYNALOOP SLIDING-PISTON LOOP

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

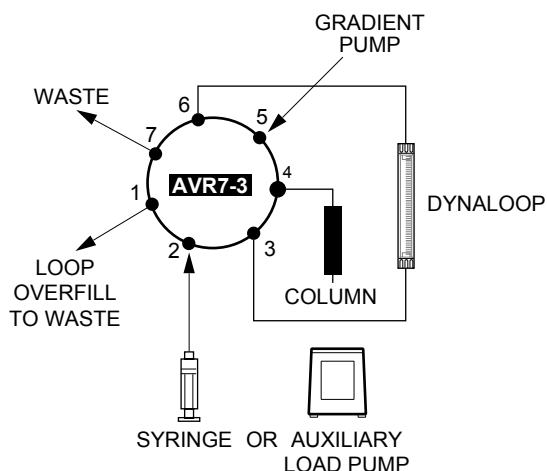
### 8.2.1 System Setup

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

The DynaLoop may be filled either manually with a syringe or automatically using an auxiliary pump such as the Econo Pump, which is controlled by the BioLogic Duo-Flow system.

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

Consult the DynaLoop user's manual or on-line help for additional information. The figure below shows how to set up a DynaLoop.



**Figure 8-3. Plumbing the DynaLoop for use with an Inject Valve**

### 8.2.2 Writing the Protocol

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

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

1. From the Manual screen, set the AVR7-3 Sample Inject Valve to its Inject (I) position and allow the sliding seal to contact the sample end fitting on the DynaLoop. Then set the valve to the Load (L) position.
2. Load sample into the DynaLoop by using a syringe. As sample is loaded into the loop, it displaces the buffer that was used to purge the unit. This design means the DynaLoop cannot be overfilled. When sufficient volume of sample for a run or series of runs is loaded into the DynaLoop, stop the filling process. For a series of partial volume injections after one loading sequence, fill the DynaLoop with about 2 percent extra sample. Leave the syringe in the Inject Port to minimize the introduction of air into the DynaLoop.  
The sample is now ready for injection, and the protocol can be programmed.
3. In the Protocol screen, select **Load/Inject Sample**. In the Load/Inject Sample window, note that the AVR7-3 valve will automatically move to the "Inject" position at the start of the step and to the "Load" position at the end of the step.
  - a. In the Load/Inject Sample window, specify Dynamic Loop.
  - b. In the "Injection Buffer" area of the window, select the buffer(s) and percent (%) composition to be used to inject the sample onto the column.
  - c. In the "Volume (ml)" field, enter the sample volume.
  - d. In the "Flow (ml/min)," enter the flow rate of the BioLogic Duo-Flow gradient pump that will be used to inject the sample onto the column.
4. Continue writing the separation protocol.

	Volume	Description	Parameters	
1	0.0	Isocratic Flow	Running Buffer Buffer B	100% 0% Volume: 10.0 ml Flow: 1.50 ml/min
2		Load/Inject Sample	<input type="radio"/> Static Loop <input checked="" type="checkbox"/> Fill before Inject <input type="checkbox"/> Rinse after Inject <input checked="" type="radio"/> Dynamic Loop <input type="checkbox"/> Fill before Inject <input type="radio"/> Direct Inject	
		Start Volume (ml)	10.0	
		Inject Sample	Auto Inject Valve will move to INJECT at Start of step and return to LOAD at end of step. Injection Buffer: Buffer A, Buffer B % Composition: A 100, B 0 Volume (ml): 25.0, Flow (ml/min): 1.50	
	35.0	End of Protocol		

Figure 8-4. Load/Inject Sample Window for a Manual Load/Inject of the Sample

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

1. Consult Section 8.3.1 for connecting the EP-1 Econo Pump as an Aux pump.
2. From the Setup screen, select Aux Load Pump and AVR7-3 valve in addition to the other items you will use to write the protocol.
3. In the Protocol screen, program an Isocratic step that is long enough for the auxiliary pump to load the desired quantity of sample into the DynaLoop. For example, if you are loading 25 ml of sample at an Aux pump flow rate of 5 ml/min, you will need at least a 5 minute step prior to the sample loading step. Otherwise the protocol will fail the automatic validation done prior to the run. The flow rate of the BioLogic Duo-Flow gradient pump is not critical, so you may set a low flow rate (e.g., 0.1 ml/min) to minimize buffer waste. However, it is important that either the time or volume length of this first step is of sufficient duration to allow the Aux pump to fill the DynaLoop.
4. In the Protocol screen, select Load/Inject Sample to program the BioLogic Duo-Flow system to automatically fill and inject the DynaLoop sample. From the Load/Inject Sample window,
  - a. Select the Dynamic Loop as the type of loop to be used.
  - b. Select Fill Before Inject. This instructs the Aux pump to load the sample into the DynaLoop. Note that the Aux pump flow rate is not under BioLogic Duo-Flow control. The flow rate is used by the system when validating the protocol before the run. Because the BioLogic Duo-Flow system only starts/stops the Aux pump, the correct flow rate must be set at the Aux pump. The flow rate of the Econo pump is set from the pump and is recorded in the yellow data entry boxes of the Fill Sample section of the Load/Inject Sample dialog box. Note that the rinse function is not available when the DynaLoop is being used.
  - c. In the Fill Sample Loop area of the window, select the sample to be loaded and enter its volume and flow rate of the Aux pump.
  - d. In the Inject Sample area of the window, select the Injection Buffers, the buffer composition, the flow rate of the gradient pump, and the sample volume to be injected onto the column.

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

5. Continue writing the separation protocol.

● BioLogic Duo Flow - <user name> - <project name> - <method name>

File Edit View Utilities Options Window Help

Method Edit Method New Run Browser Report Manual Setup Protocol Run Notes PostRun Log Cut Copy Paste Delete

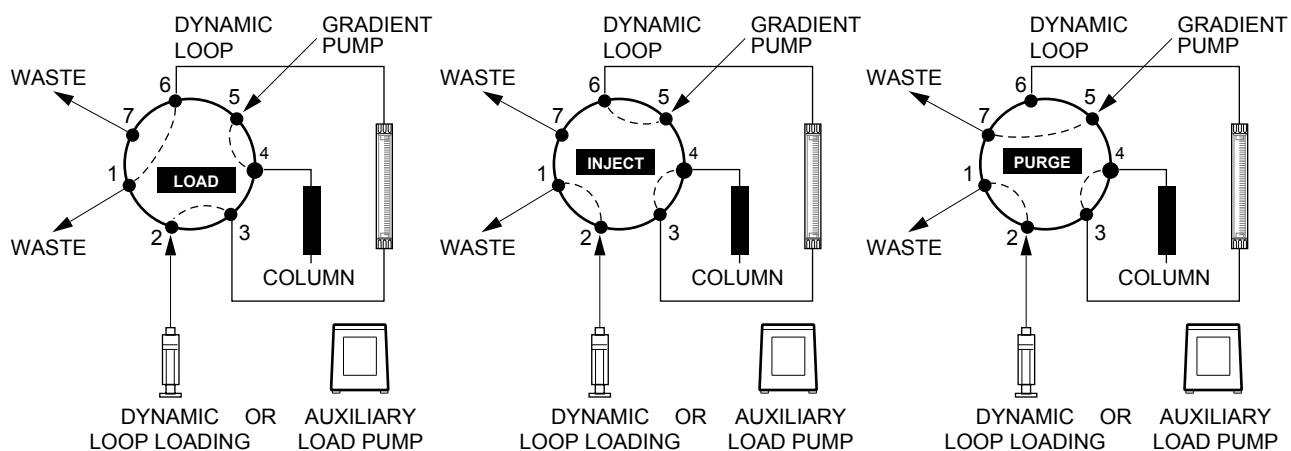
Add Step

Volume	Description	Parameters	
1	Isocratic Flow	Running Buffer 100%	Volume: 10.0 ml
		Buffer B 0%	Flow: 1.50 ml/min
2	Load/Inject Sample	<input type="radio"/> Static Loop <input checked="" type="checkbox"/> Fill before Inject <input type="checkbox"/> Rinse after Inject <input type="button" value="Ok"/> <input checked="" type="radio"/> Dynamic Loop <input checked="" type="checkbox"/> Fill before Inject <input type="button" value="Cancel"/> <input type="radio"/> Direct Inject <input type="text" value="Aux Pump Flow Rate not under BioLogic Control"/>	
	Start Volume (ml)	10.0	
	Fill Sample Loop	Sample: <input type="text" value="Sample"/> Volume (ml) <input type="text" value="25.0"/> Flow (ml/min) <input type="text" value="1.00"/>	
	Inject Sample	Injection Buffers: <input type="text" value="Buffer A"/> <input type="text" value="Buffer B"/> % Composition: A <input type="text" value="100"/> B <input type="text" value="0"/> Volume (ml) <input type="text" value="25.0"/> Flow (ml/min) <input type="text" value="1.50"/>	
	Auto Inject Valve will move to INJECT at Start of step and return to LOAD at end of step.		
60.0	End of Protocol		

Figure 8-5. Load/Inject Sample Window for an Automatic Load/Inject of the Sample

During the run, the valve functions as follows:

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



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

### 8.3 SAMPLE LOADING ONTO A LOW PRESSURE COLUMN USING THE ECONO PUMP AS AN AUX PUMP

The EP-1 Econo Pump is ideal for loading samples onto a low-pressure column. However, when using a low-pressure column, such as an Econo-Pac cartridge, remove the 40-psi back-pressure device from the post-column position. Place it between the Workstation's Gradient Pump and the injection valve. This will allow the check valves to properly seat and ensure pump flow performance. In addition, low pressure columns may burst if the back pressure device is placed after the column.

#### 8.3.1 System Setup

The BioLogic Duo-Flow can be used with the following auxiliary pumps:

- Model EP-1 Econo Pump.

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

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

**Note:** The BioLogic Duo-Flow system does not control the flow of the Econo Pump. Because the BioLogic Duo-Flow system only starts/stops the Aux pump, the correct flow rate must be set at the Aux pump.

- Other external pumps.

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

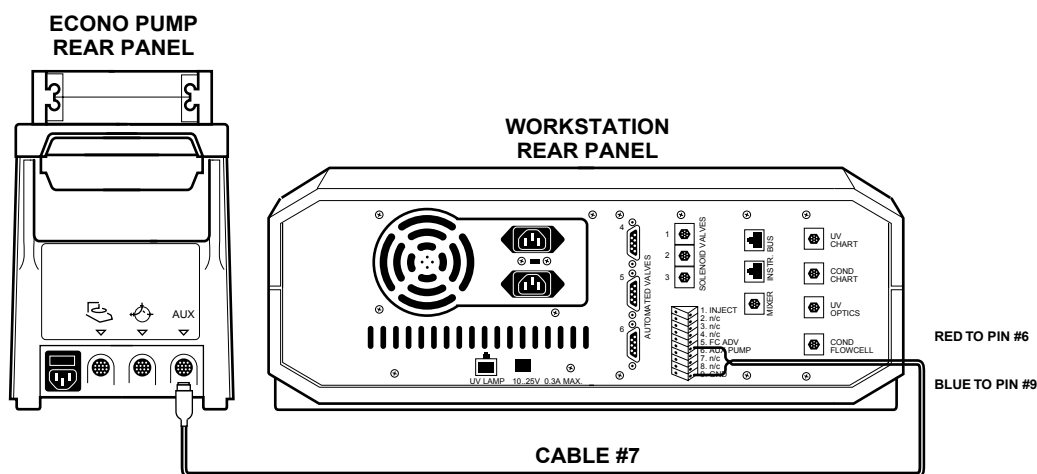


Figure 8-7. Connecting an EP-1 Econo Pump to the BioLogic Duo-Flow Workstation

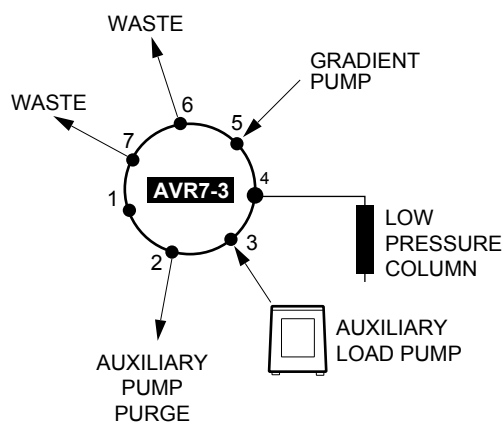
To connect the Econo Pump with the BioLogic Duo-Flow, make the following cable connections:

1. Obtain **System Cable #7**, Catalog # 731-8267.
2. Locate the red and blue wires at the wire end of the cable and connect them to the BioLogic Duo-Flow Workstation.
 

**Note:** Cut short all other wires and isolate with tape.

  - a. Connect the red wire to pin 6 of the Aux connector.
  - b. Connect the blue wire to pin 9 of the Aux connector.
3. Plug the 8-pin mini-DIN connector into the Aux connector on the rear panel of the Econo Pump.

When plumbing the Econo Pump, be sure to use PharMed tubing. For additional information on the Econo Pump, consult its user manual. The AVR7-3 injection valve is plumbed without a sample loop. Plumb the valve as shown below, with the EP-1 Econo Pump connected to port 3 of the Inject Valve.



**Figure 8-8. Plumbing an AVR7-3 Inject Valve with an EP-1 Econo Pump as an Auxiliary Load Pump**

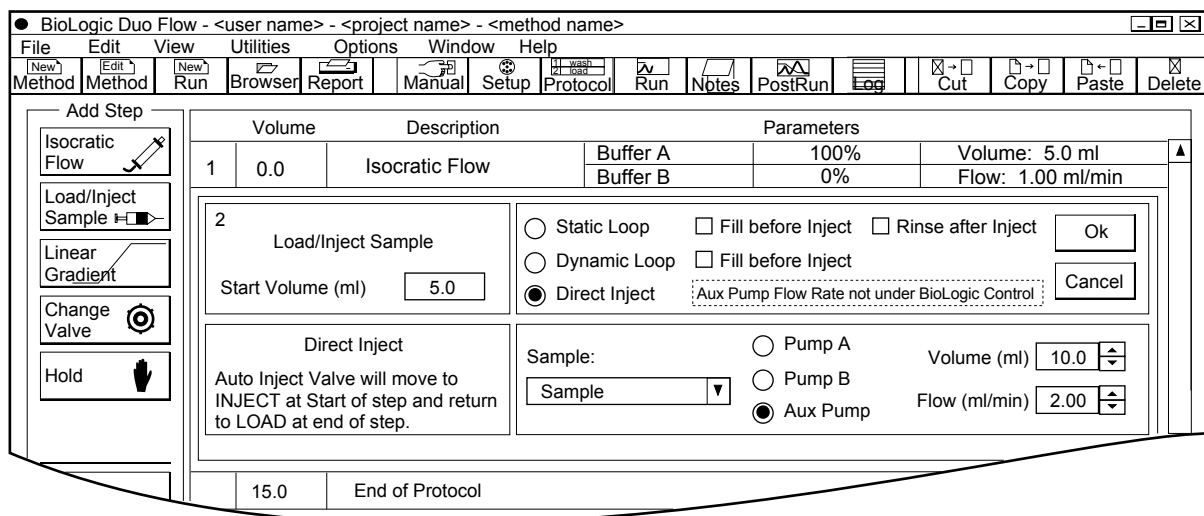
In the Setup screen, select the AVR7-3 valve as the Sample Inject Valve and add the Aux Pump to the Setup List in addition to the other items you will use to write the protocol. When using an auxiliary pump to load sample directly to an AVR7-3 Sample Inject valve, it is possible to have a valve, such as an SVT3-2 valve, assigned as the “Aux Pump Inlet” to allow switching between samples or rinse solutions.

### 8.3.2 Writing the Protocol

The following steps should be programmed within the Method Protocol, as shown in the next figure:

1. At the Load/Inject Sample step, specify Direct Inject and choose Aux Pump as the inlet through which the sample will be delivered. Note the message displayed in yellow, “Aux Pump Flow Rate not under Biologic control,” and that the Flow rate also is displayed in yellow. This is to serve as a reminder that the BioLogic Duo-Flow only starts/stops the Aux pump and that the actual flow rate must be set at the Aux pump.
2. Enter the volume of sample and the flow rate for the injection step. The step starting time or volume appears to the left of the options buttons.
 

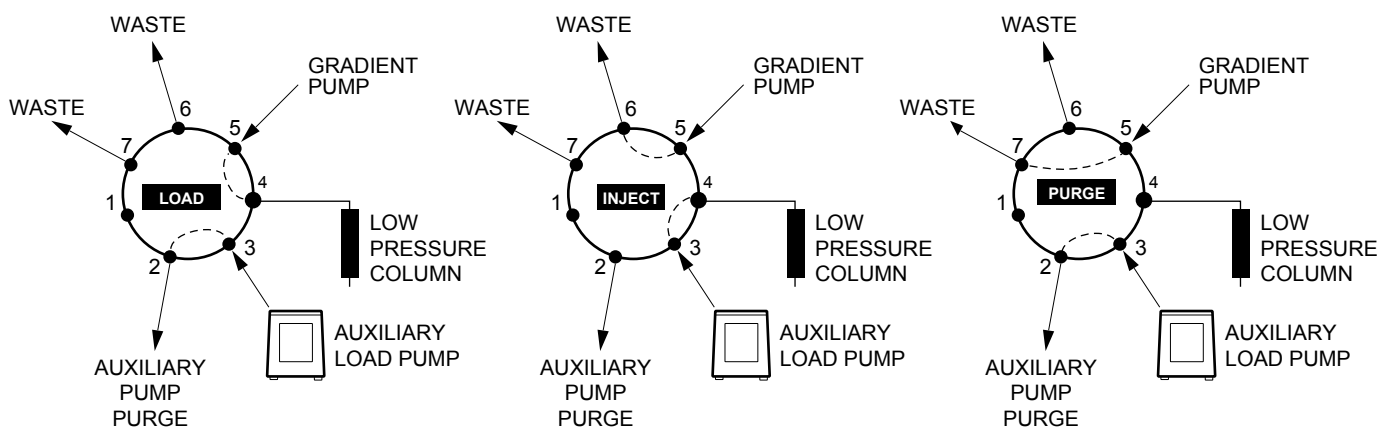
**Note:** The BioLogic Duo-Flow system does not control the flow of the Econo Pump. The correct flow rate must be entered at the Econo Pump.
3. Continue writing the separation protocol.



**Figure 8-9. Writing the Protocol: Direct Sample Loading**

In the configuration shown below, the valve operates as follows:

- **LOAD.** While in this position the valve connects ports 5 and 4 for equilibration of the column and for sample elution.
- **INJECT.** While in this position the valve connects ports 3 and 4 to load sample onto the column.
- **PURGE.** While in this position the valve connects ports 5 and 7 and allows purging or buffer changes without the need to remove the column from the system.



**Figure 8-10. Valve Positions During a Run with Direct Sample Loading and Injection**

## 8.4 LOADING MULTIPLE SAMPLES

With the addition of the Econo Pump as an Aux pump and an AVR9-8 as an Aux pump inlet valve, the system can load up to 7 samples sequentially.

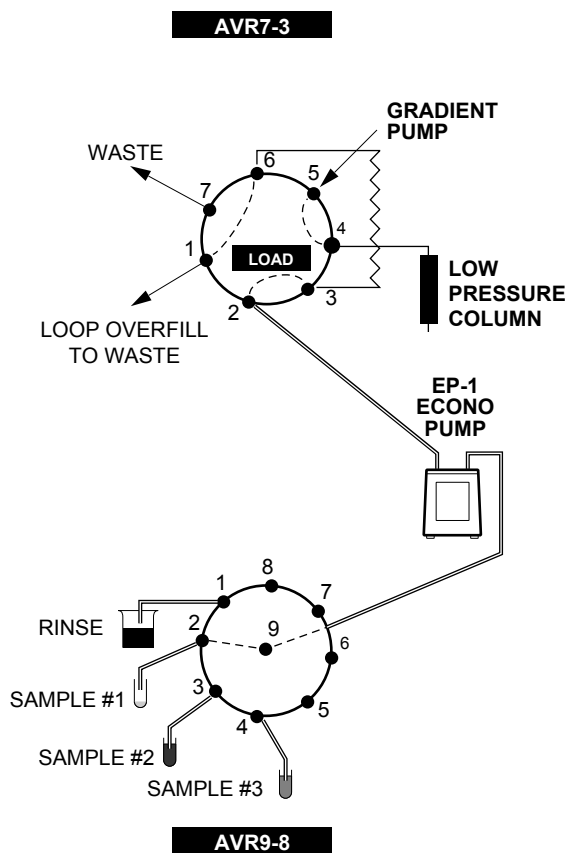
### 8.4.1 System Setup

Connect the Econo Pump as described in section 8.3.1. In the Setup screen, alias the AVR9-8 as an Aux Pump Inlet. Name seven positions to identify each sample, and name one position "Rinse." The rinse solution will clean the sample loop between injections. Add the Aux pump to the Setup List.

**Note:** Some sample will be lost in the process of pulling sample from a remote beaker. Be sure to factor in the volume that is required to fill all buffer lines leading up to the top of the column when programming the injection step.

When using a low-pressure column such as an Econo-Pac cartridge, remove the back-pressure device from a post-column position. Place it between the pump and injection valve. This will allow the check valves to properly seat. In addition, low-pressure columns may burst if the back-pressure device is placed after the column.

See the illustration below to assist in setting up the system. Be sure to keep tubing lengths to a minimum when plumbing the system. This will minimize sample loss.



**Figure 8-11. Using a Peristaltic Pump and an AVR9-8 Valve to Load Multiple Samples**



### 8.4.2 Writing the Protocol

The following steps should be programmed within the method protocol:

1. Remember that the Econo Pump flow rate is not under BioLogic Duo-Flow control. Because the BioLogic Duo-Flow system only starts/stops the Aux pump, the correct flow rate must be set at the Aux pump. Set the correct flow rate on the Econo Pump and enter the flow rate in the yellow boxes of the Load/Inject Sample dialog box.
2. If multiple injections are performed in a single Run, you may view or print each separation independently by opening the Post-Run window and highlighting the section of the chromatogram you wish to view.
3. At the Load/Inject Sample step, specify Static Loop and choose "Fill before Inject" and "Rinse after Inject." This automates the process of filling the sample loop using the Aux pump, injecting the sample using the gradient pump, and then rinsing the sample loop using the Aux pump. Note the message displayed in yellow, "Aux Pump Flow Rate not under BioLogic control," and that the Flow rates also are displayed in yellow. This is to serve as a reminder that the actual flow rate must be set at the Aux pump. Refer to the figure below.
4. Continue writing the separation protocol.

Volume	Description	Parameters
1	Isocratic Flow	Buffer A: 100% Buffer B: 0% Volume: 5.0 ml Flow: 1.00 ml/min
2	Load/Inject Sample	Start Volume (ml): 5.0 Static Loop (selected) <input checked="" type="checkbox"/> Fill before Inject <input checked="" type="checkbox"/> Rinse after Inject Dynamic Loop <input type="checkbox"/> Fill before Inject Direct Inject Aux Pump Flow Rate not under BioLogic control Fill Sample Loop: Sample: Sample 1 Volume (ml): 5.0 Flow (ml/min): 5.00 Inject Sample: Injection Buffers: Buffer A, Buffer B % Composition: A 100%, B 0% Volume (ml): 10.0 Flow (ml/min): 2.00 Rinse Sample Loop: Rinse Buffer: Rinse Pump A, Pump B, or Aux Pump (selected) Volume (ml): 5.0 Flow (ml/min): 5.00
20.0	End of Protocol	Volume includes 5.0 ml for Rinse after Sample Inject

**Figure 8-12. Load/Inject Sample Window for Loading Multiple Samples**

## 8.5 MULTIPLE INJECTIONS OF A SINGLE SAMPLE

The BioLogic Duo-Flow system can be used in conjunction with the DynaLoop to run repeated injections of the same sample.

### 8.5.1 System Setup

Install the DynaLoop (either the 25 ml or 90 ml loop) as described in section 8.2.

### 8.5.2 Writing the Protocol

The following should be programmed within the method protocol:

1. In the Load/Inject Sample step, choose Dynamic Loop and enter the volume of sample to be injected during each run. At the initiation of the run, select Multiple Runs and the number of times you want to repeat the separation.  
**Note:** The BioLogic Duo-Flow system does not sense how much sample is available in the sample loop, and will not indicate a programming error if you program more runs than you have sample for.
2. Continue writing the separation protocol.

## 9.0 MULTIPLE COLUMNS

The BioLogic Duo-Flow system supports the use of multiple columns. Refer to Chapter 2, section 2.6, Columns and Column Fittings, for a description of columns and fittings offered by Bio-Rad. Several examples are discussed below.

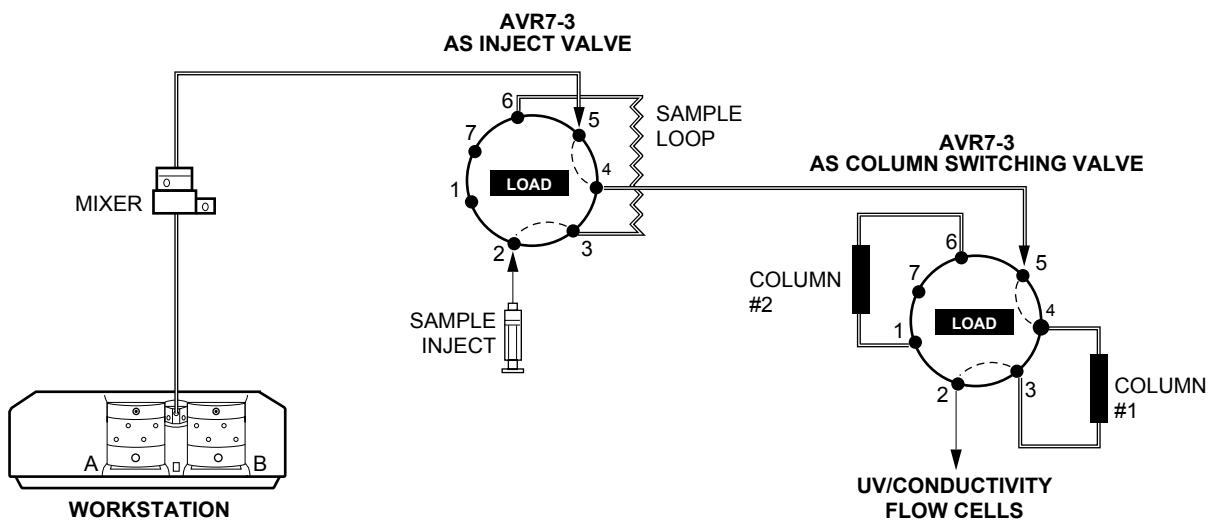
### 9.1 COLUMN SWITCHING

For this application two AVR7-3 valves will be used: one as a sample injector, and the second used down stream as a column switcher. Alternatively, an SV5-4 valve may be used for sample loading (refer to section 8.1) and one AVR7-3 valve for column switching.

#### 9.1.1 System Setup

Figure 9-1 below shows the two valve positions when the injection valve is used for column switching. In this configuration, the sample from an upstream injection valve enters via port 5 and is diverted to either column using the Load and Inject positions of the valve. Although the purge position is not described in this application, it may be used in the conventional manner.

On the Column switching valve, Column 1 has its inlet connected to port 4 and its outlet to port 3. Column 2 has its inlet connected to port 6 and its outlet to port 1. Port 5 functions as a common inlet port, and port 2 functions as a common outlet port.



**Figure 9-1. Column Switching using Two AVR7-3 Valves**

In the Setup screen, use the default name of "Sample Inject" for the first AVR7-3 valve. Name the second AVR7-3 "column switcher." Enter the column names into positions 1 and 2, and name position 3 as "Purge."

Note that when solution is directed to Column 1, this is similar to the "Load" position. When solution is directed to Column 2, this is similar to the "Inject" position.

### 9.1.2 Writing the Protocol

The following steps should be programmed within the Method Protocol, as shown in the figure below.

1. Equilibrate both columns with the appropriate equilibration buffer.
2. Use the "Change Valve" step to ensure that the column switching valve is in the appropriate position for loading the sample onto either Column 1 or Column 2.
3. Use the Load/Inject Sample step to load half the sample on to the appropriate column and continue to write the separation protocol for the sample and column.
4. Use the "Change Valve" step to ensure that the column switcher valve is in the appropriate position for loading sample onto either Column 1 or Column 2.
5. Use precautionary washes/rinses to prevent cross-contamination by selecting the purge position on the column switcher valve. This is used to clean the tubing between the two AVR7-3 valves. Alternatively, performing an isocratic flow step before directing flow to the other column may also be used. This later step is depicted in the figure below.
6. Continue writing the separation protocol.

BioLogic Duo-Flow - <user name> - <project name> - <method name>

File Edit View Utilities Options Window Help

New Method Edit Method New Run Browser Report Manual Setup Protocol Run Notes PostRun Log Cut Copy Paste Delete

Add Step

Isocratic Flow Load/Inject Sample Linear Gradient Change Valve Hold Alarm Zero Baseline UV Lamp Chart Recorder Repeat Steps Fraction Collection	1	0.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
				Buffer B	0%	Flow: 1.00 ml/min
	2	5.0	Change Valve	Column Switcher	Position: Column #1	
	3	5.0	Load/Inject Sample	Load: Sample		Volume: 1.0 ml
				Static Loop	Auto Inject Valve	Flow: 2.00 ml/min
	4	6.0	Linear Gradient	Buffer A	100% --> 30%	Volume: 6.0 ml
				Buffer B	0% --> 70%	Flow: 1.00 ml/min
	5	12.0	Isocratic Flow	Buffer A	0%	Volume: 10.0 ml
				Buffer B	100%	Flow: 2.00 ml/min
	6	22.0	Isocratic Flow	Buffer A	100%	Volume: 10.0 ml
				Buffer B	0%	Flow: 2.00 ml/min
	7	32.0	Change Valve	Column Switcher	Position: Column #2	
8	32.0	Isocratic Flow	Buffer A	100%	Volume: 10.0 ml	
			Buffer B	0%	Flow: 2.00 ml/min	
9	42.0	Load/Inject Sample	Load: Sample		Volume: 1.0 ml	
			Static Loop	Auto Inject Valve	Flow: 2.00 ml/min	
10	43.0	Linear Gradient	Buffer A	100% --> 30%	Volume: 6.0 ml	
			Buffer B	0% --> 70%	Flow: 1.00 ml/min	
11	49.0	Isocratic Flow	Buffer A	0%	Volume: 10.0 ml	
			Buffer B	100%	Flow: 2.00 ml/min	
12	59.0	Isocratic Flow	Buffer A	100%	Volume: 10.0 ml	
			Buffer B	0%	Flow: 2.00 ml/min	
	69.0	End of Protocol				

Gradient Pump UV Conductivity Aux. Pump SIM1/SIG SIM1/pH

0.00ml/min 0 %B 0 psi 0.2869 AU 29.4 mS/cm Stopped -0.000 Volt 7.00 pH

Select item(s) for copy/cut/delete. Double-click on step to edit.

Figure 9-2. Protocol for using an AVR7-3 Inject Valve for Column Switching

## 9.2 RUNNING ONE SAMPLE SEQUENTIALLY OVER SEVERAL DIFFERENT COLUMNS

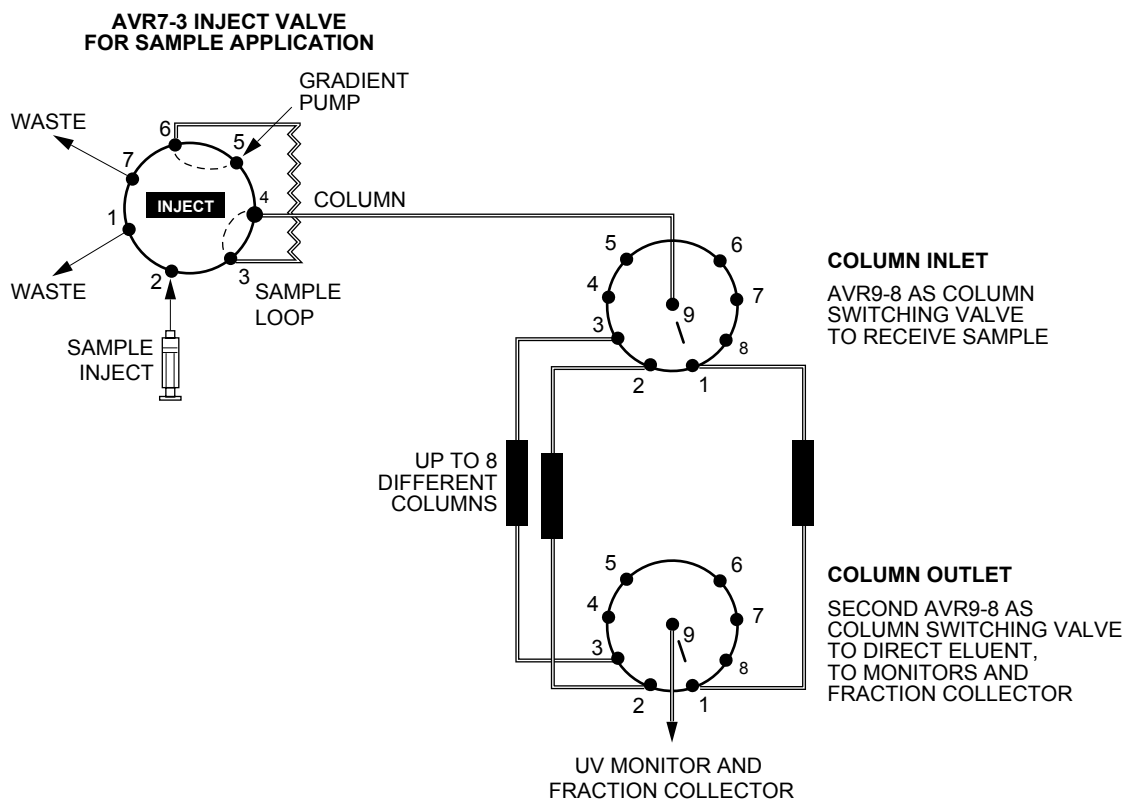
With the addition of two AVR9-8 valves, up to eight columns can be tested with a single sample.

### 9.2.1 System Setup

In the Setup screen, select "User-assigned name" when naming each of the AVR9-8 valves. Name the first AVR9-8 valve "column inlet," and type in the names of the columns in positions 1-8. Name the second AVR9-8 valve "column outlet," and type in the names of the columns in positions 1-8.

The two column switching valves will be plumbed after the AVR7-3 inject valve. Position both valves to a convenient location on the rack to minimize tubing lengths. Connect port 4 of the inject valve to the common port of the "column inlet" valve. Connect the common port of the "column outlet" valve to the UV flow cell.

As with plumbing any column, be certain that all column inlet lines are free of air to avoid introducing air into the columns.



**Figure 9-3. Column Switching using Two AVR9-8 Valves**

## 9.2.2 Writing the Protocol

The following should be programmed within the Method Protocol:

1. Begin the protocol by adding two Change Valve steps: one to position the column inlet at starting position, and the second to position the column outlet at the starting position. Be sure that these two positions are identical. For example if you wish to equilibrate column #1 with buffer A, the protocol should look as shown in the figure below.

**Note:** Lower the flow rate to less than or equal to 1.0 ml/min see steps 8 and 14 below) when performing sequential "Change Valve" steps.

2. Continue writing the separation protocol.

If multiple injections are performed in a single run, you may view or print each separation independently open up post run and highlight the section of the chromatogram you wish to view.

BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File Edit View Utilities Options Window Help						
Method Method Run Browser Report Manual Setup Protocol Run Notes PostRun Log Cut Copy Paste Delete						
Add Step						
<div>Isocratic Flow</div> <div>Load/Inject Sample</div> <div>Linear Gradient</div> <div>Change Valve</div> <div>Hold</div> <div>Alarm</div> <div>Zero Baseline</div> <div>UV Lamp</div> <div>Chart Recorder</div> <div>Repeat Steps</div> <div>Fraction Collection</div>	1	0:00.0	Change Valve	column inlet	Position: Column 1	
	2	0:00.0	Change Valve	column outlet	Position: Column 1	
	3	0:00.0	Isocratic Flow	Buffer A	100%	Time: 10.0 min
				Buffer B	0%	Flow: 2.00 ml/min
	4	10.0	Load/Inject Sample	Load: Sample	Volume: 6.0 ml	
				Static Loop	Auto Inject Valve	Flow: 2.00 ml/min
	5	13.0	Linear Gradient	Buffer A	100% --> 30%	Time: 6.0 min
				Buffer B	0% --> 70%	Flow: 1.00 ml/min
	6	19.0	Isocratic Flow	Buffer A	0%	Time: 5.0 min
				Buffer B	100%	Flow: 2.00 ml/min
	7	24.0	Isocratic Flow	Buffer A	100%	Time: 5.0 min
				Buffer B	0%	Flow: 2.00 ml/min
	8	29.0	Isocratic Flow	Buffer A	100%	Time: 0.1 min
				Buffer B	0%	Flow: 1.0 ml/min
	9	29.1	Change Valve	column inlet	Position: Column 2	
	10	29.1	Change Valve	column outlet	Position: Column 2	
	11	29.1	Load/Inject Sample	Load: Sample	Volume: 6.0 ml	
				Static Loop	Auto Inject Valve	Flow: 2.00 ml/min
	12	32.1	Linear Gradient	Buffer A	100% --> 30%	Time: 6.0 min
				Buffer B	0% --> 70%	Flow: 1.00 ml/min
	13	38.1	Isocratic Flow	Buffer A	100%	Time: 10.0 min
				Buffer B	0%	Flow: 2.00 ml/min
	14	48.1	Isocratic Flow	Buffer A	100%	Time: 1.0 min
				Buffer B	0%	Flow: 1.0 ml/min
	15	48.2	Change Valve	column inlet	Position: Column 3	
	16	48.2	Change Valve	column outlet	Position: Column 3	
	17	48.2	Isocratic Flow	Buffer A	100%	Time: 10.0 min
				Buffer B	0%	Flow: 2.00 ml/min
		58.2	End of Protocol			

Figure 9-4. Programming a Sequential Run

## 10.0 REVERSE FLOW CHROMATOGRAPHY

Using two AVR7-3 inject valves for reverse flow chromatography is advantageous for certain applications such as affinity chromatography, where minimal dilution during sample elution is important.

### 10.1 SYSTEM SETUP

In the Setup screen, select one AVR7-3 valve as the Sample Inject valve. Select a second AVR7-3 valve and name (alias) it as "Change Flow Valve." On the "Change Flow Valve," alias position 1 as Forward Flow, and alias position 2 as Reverse Flow. Leave Position 3 as is.

Connect the sample loop to ports 3 and 6 of the AVR7-3 inject valve. To the Change Flow valve, connect the column inlet to port 6, the column outlet to port 4. The Change Flow valve will require a connection between ports 1 and 2. Use approximately 8 cm of 1/16" (1.6 mm) OD tubing to make this connection. Keep this tubing length to a minimum to minimize sample dilution. Use minimal length of 1/16" (1.6 mm) OD tubing to connect port 4 of the AVR7-3 inject valve to port 5 of the Change Flow valve. Figure 10-1 shows how to set up this configuration.

If a low-pressure column is used, remove the 40-psi backpressure device and place it between the pump and the AVR7-3 valve. This will allow the check valves to properly seat and ensure pump flow performance. In addition, low-pressure columns will burst if the back-pressure device is placed after the column.

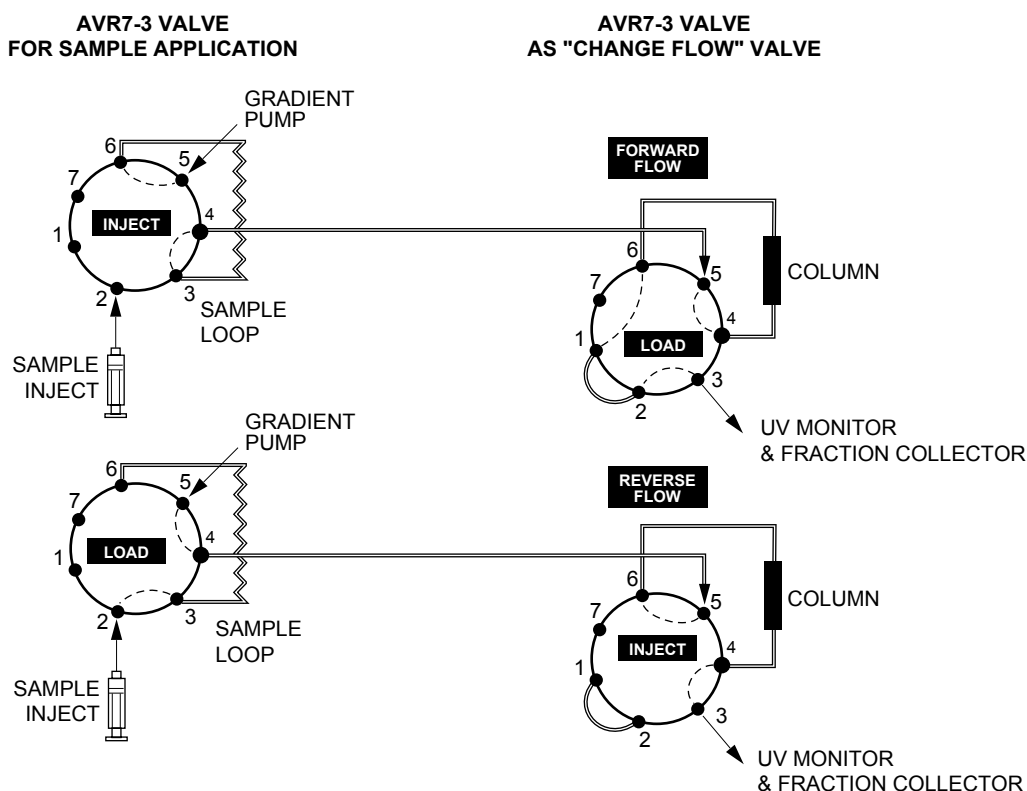


Figure 10-1. Injection Valve Plumbing for use in Affinity Chromatography

## 10.2 WRITING THE PROTOCOL

The following steps should be programmed within the method protocol.

1. Ensure that the column is properly equilibrated by using an Isocratic step.
2. Use a Change Valve step to place the Change Flow Valve in the Forward Flow orientation.
3. Use the Load/Inject Sample step to load the sample loop and subsequently inject it onto the column. The AVR7-3 valve will automatically move to the INJECT position at the start of the step and return to the LOAD position at the end of the step.
4. Use the appropriate buffer to wash all nonbinding components from the column.
5. When the sample is to be eluted, first use the Change Valve step to select the "Reverse Flow" valve position of the Change Flow valve. This directs the flow of buffer through the bottom of the column to elute sample components of interest in a concentrated form.
6. Continue writing the separation protocol.

● BioLogic Duo-Flow - <user name> - <project name> - <method name>

File Edit View Utilities Options Window Help

New Edit Run Browser Report Manual Setup Protocol Run Notes PostRun Log Cut Copy Paste Delete

Add Step

Isocratic Flow

Load/Inject Sample

Linear Gradient

Change Valve

Hold

Alarm

Zero Baseline

UV Lamp

Chart Recorder

Repeat Steps

Fraction Collection

1	0.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
			Buffer B	0%	Flow: 1.00 ml/min
2	5.0	Change Valve	Change Flow Valve	Position: Forward Flow	
3	5.0	Load/Inject Sample	Load: Sample		Volume: 1.0 ml
			Static Loop	Auto Inject Valve	Flow: 2.00 ml/min
4	6.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
			Buffer B	0%	Flow: 1.00 ml/min
5	11.0	Change Valve	Change Flow Valve	Position: Reverse Flow	
6	11.0	Linear Gradient	Buffer A	100% --> 30%	Volume: 3.0 ml
			Buffer B	0% --> 70%	Flow: 1.00 ml/min
7	14.0	Isocratic Flow	Buffer A	0%	Volume: 1.0 ml
			Buffer B	100%	Flow: 1.00 ml/min
8	15.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
			Buffer B	0%	Flow: 1.00 ml/min
9	20.0	Change Valve	Change Flow Valve	Position: Forward Flow	
	20.0	End of Protocol			

Gradient Pump			UV	Conductivity	Aux. Pump	SIM1/SIG ▼	SIM1/pH ▼
0.00ml/min	0 %B	0 psi	0.2869 AU	29.4 mS/cm	Stopped	-0.000 Volt	7.00 pH

Select item(s) for copy/cut/delete. Double-click on step to edit.

Figure 10-2. Programming for Reverse Flow Chromatography



## 11.0 MAINTENANCE

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The BioLogic Duo-Flow system requires minimal maintenance to assure reliable operation.

### 11.1 CLEANING THE OUTER SURFACES OF THE INSTRUMENTS

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

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

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

### 11.2 STORAGE OF THE BIOLOGIC DUO-FLOW SYSTEM

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

1. The system tolerates low salt buffer overnight, but sample and high salt buffers need to be washed out of the tubing.
2. Use DI water and the 10mL syringe provided in the System Fittings Kit to wash behind each pump head with at least 10mL of water. This applies to both the F-10 and F-40 pumps.
3. If the system is in a cold environment, keep it powered on to prevent condensation. If it is at room temperature, it may be left off or on. (When it is at room temperature, the BioLogic Duo-Flow Color Monitor can be turned off, to conserve energy and prolong monitor life.)
4. Thoroughly wash all valves, especially the SVT3-2 and SV5-4 solenoid valves.

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

1. Remove the column from on-line. Follow the column manufacturer's instructions to clean and store the column.
2. Run DI water through the entire system. Be sure to wash through all parts of both the solenoid and the automated valves to remove any contaminants.  
**Note:** It is particularly important to wash out solenoid valves. Failure to do so may result in costly replacements.
3. Use DI water and the 10mL syringe provided in the System Fittings Kit to wash behind each pump head with at least 10 mL of water. This applies to both the F-10 and F-40 pumps.
4. Thoroughly wash all valves, especially the SVT3-2 and SV5-4 solenoid valves.
5. Store the entire system with either 20% EtOH or 0.05% sodium azide to inhibit bacterial growth. If necessary, fit the pump inlets, UV and conductivity monitors, and all solenoid valves with 1/4-28 plugs which are provided in the System Fittings Kit.

### 11.3 CARE AND MAINTENANCE OF THE GRADIENT PUMP

These following three sections describe the procedures for priming to remove air bubbles and periodic maintenance of the Gradient Pumps.

#### 11.3.1 Priming the Gradient Pump and Removing Trapped Air Bubbles

**Warning:** Do not run the Gradient Pumps dry (without buffers in line), as this may result in damage to the pump heads.

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

1. Check that both of the inlet tubings are securely placed into buffer bottles A and B. Inlet tubing should be finger-tightened. Inlet filters are highly recommended.
2. Remove air from inlet buffer lines and prime the Gradient Pump.

**Note:** Air bubbles trapped behind the pump head pistons will not damage the pump head but will produce an erratic flow. This problem is easily diagnosed by watching the pressure readout on any screen in the BioLogic software status bar. If it is extremely erratic and unstable, then suspect a trapped bubble and repeat the above steps.

Some pressure fluctuation ( $\pm 10\%$ ) is normal due to the response of the pressure transducer over the available flow rate of the Gradient Pump.

- a. Insert a 10 ml syringe into the luer fitting of the priming port (see Figure 4-2) for Pump A of the F-10 or F-40 pumps. Open the priming valve by rotating it counterclockwise. Draw buffer into the inlet buffer line and into the syringe. Close the priming valve (rotate clockwise) prior to removing the syringe, and expel the buffer into a waste container.
- b. Repeat several times until no more bubbles are seen and then fill the syringe with 10 ml of buffer. Close the priming valve and remove air from the syringe by tapping the sides of the syringe to bring air bubbles to the top.
- c. Remove the Pump A outlet tubing (See Figure 4-2). Attach the syringe filled with buffer A to the priming port and open the priming valve. Cover the pump A outlet with a paper towel to catch expelled buffer. Pulse the syringe plunger vigorously to expel any bubbles trapped behind the pump head piston. Do not push all of the buffer into the pump because air might be introduced in the last milliliter of buffer. Tighten the outlet fitting and close the priming port while the syringe is still in place.
- d. Repeat for Pump B.

#### 11.3.2 Daily Maintenance

It is recommended that an SVT3-2 diverter valve be set up between Buffer B and Pump B for automatically flushing the system. Use one of the valve input ports to run the buffer and use the other input port to run water through the pump head, rinsing it of salts after each run. Refer to Chapter 4, Figure 4-9.

Washing behind the piston seal extends seal life. Insert a 10 ml syringe filled with deionized water in the Washout Port luer fitting at the top of pump head. A 10-ml syringe is provided in the BioLogic Duo-Flow System Fittings Kit. Each pump head should get a 10 ml washout at the end of the day's operation. Run off water exits through the Washout Drain between the two pump heads. It can be collected in a small beaker.

### 11.3.3 Routine Maintenance of the Gradient Pump

The system Gradient Pump requires minimal maintenance to stay in good working condition. The Duo-Flow F-10 Pump and F-40 Pump maintenance kits include the piston seals, check valves, and O-rings for routine maintenance of the pumps. Indications that maintenance is required include flow rates that are below the specified rate, an erratic delivery of liquid, liquid running out of the Washout drain during normal operation (often manifested as salt crystal accumulation), and poor gradient performance.

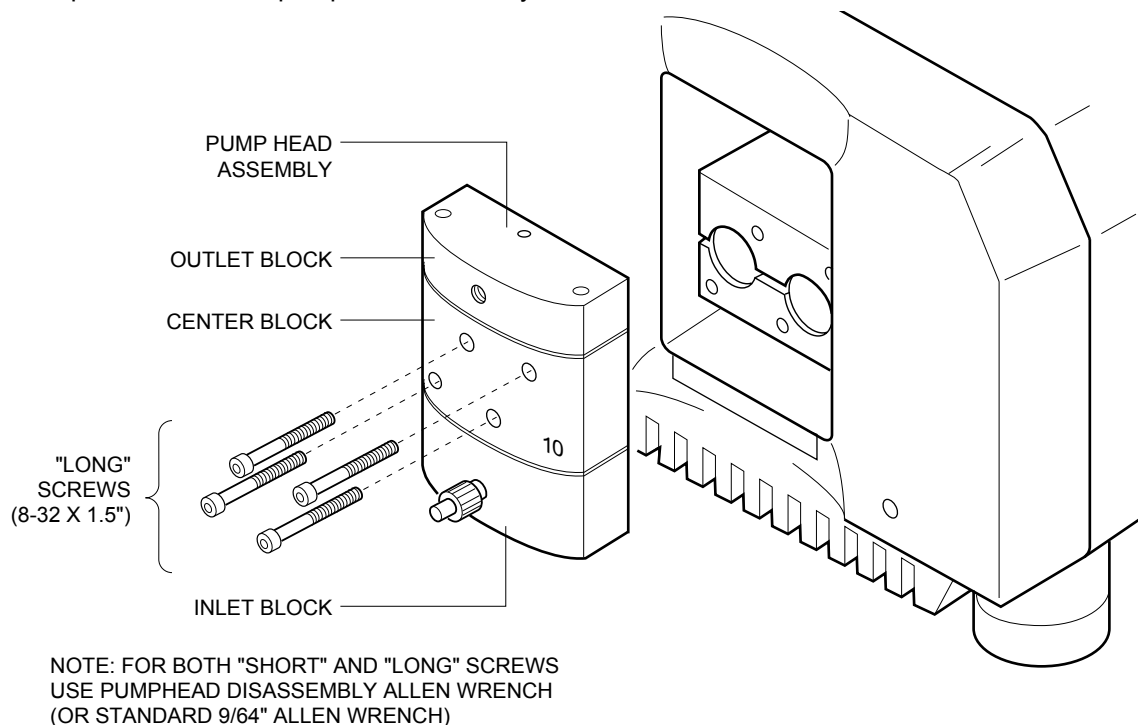
**Safety:** Make sure that any hazardous material has been flushed from the system, the pumps are not running, and any residual pressure has been bled from the system. Disconnect the Workstation power. Wear eye protection, gloves, and other protection as appropriate.

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

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

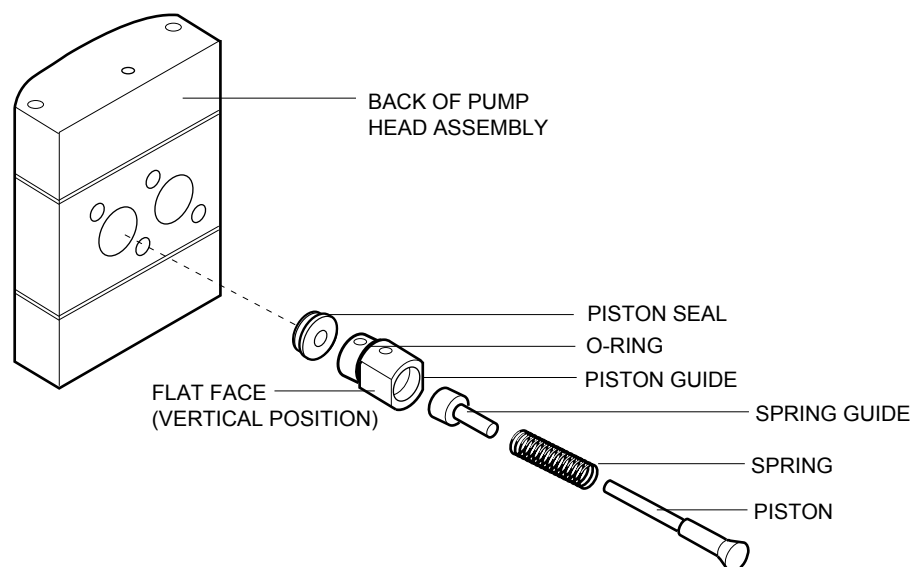
This procedure will require new piston seals. See Appendix D, Ordering Information.

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



**Figure 11-1. Workstation Pump Mechanism Parts**

- e. Note the piston seal in each piston well. Always replace both seals at the same time. Using the Piston Seal Removal Tool, pry the seal from the piston well. To do this, you insert the tip of the Seal Removal Tool into the seal and gently pry the seal away. Once a piston seal is removed, it cannot be reused.



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

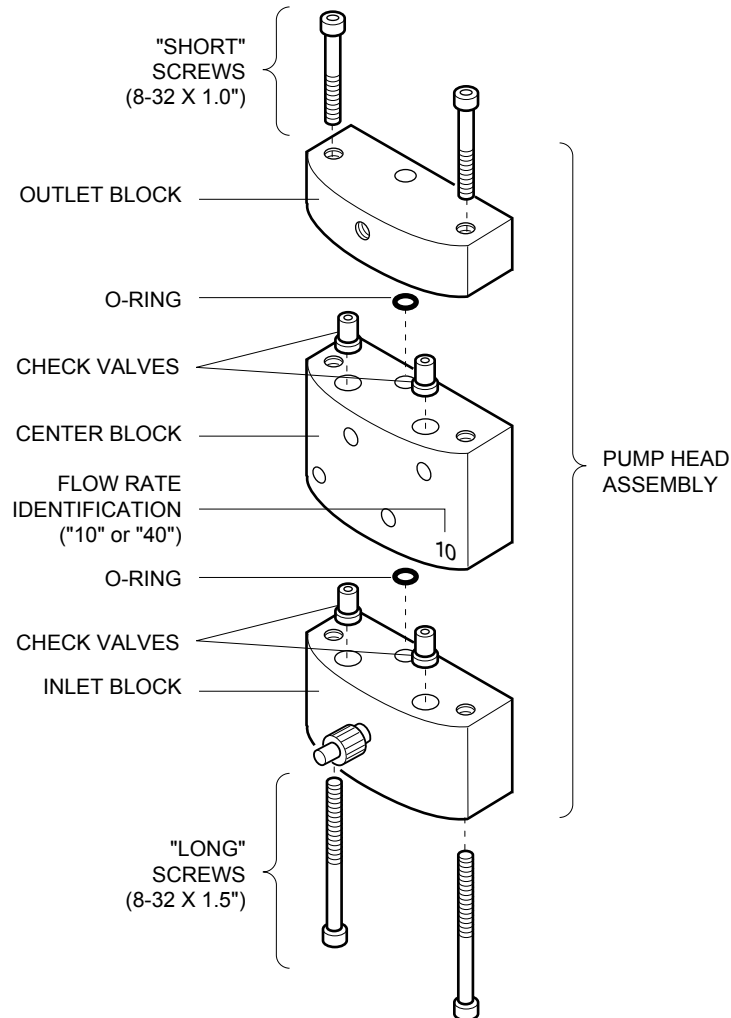
- f. Using your finger, press each new piston seal into its recess. Use the piston guide to press the seal completely into its recess. Condition the seal by pumping 100 ml of 100% methanol through each pumphead with the 40-psi backpressure device in place.
- g. Wipe the pistons with a dampened cloth before returning them to the piston wells. This ensures that no crystallized salt residues are present which could damage the new seals.  
When returning each piston assembly to its piston well, make sure that the seals in the piston wells on the pump head assembly are securely mounted in place. The piston assemblies should be oriented so that the flat faces on the piston guides are in the vertical position.
- h. Using the four long mounting screws removed earlier, remount the pump head module to the Workstation so that it is secure. Insert and tighten the mounting screws. **Do not over tighten.**
- i. Flush the system with water before running aqueous buffers.

## 2. Cleaning and replacing a check valve.

- a. Remove the two short screws at the top of the pump head assembly and the two long screws at the bottom of the pump head assembly. Each pump separates into three blocks, revealing four check valves and two O-rings.
- b. Replace the check valves. It is not necessary to replace the O-rings, as they are not subjected to high pressure.
- c. Reassemble the pump head. (Refer to Figure 11-3.) Check valves fit into the pump head module in one direction only.
- d. Wipe the pistons with lab tissue before returning them to the piston wells. This ensures that no crystallized salt residues are present which could damage the new seals.  
The piston assemblies should be oriented so that the flat faces on the piston guides are in the vertical position.
- e. Using the four long mounting screws removed earlier, remount the pump head assembly to the Workstation so that it is secure. Insert and alternately tighten the mounting screws. Do not over tighten.

### 3. Care of the Pistons.

To maintain pump performance, the pistons should be rinsed on a nightly basis. This may easily be done automatically by programming a water rinse step at the end of each protocol. This may also be done manually by washing the pistons with the syringe provided in the Fittings Kit.



NOTE: FOR BOTH "SHORT" AND "LONG" SCREWS  
USE PUMPHEAD DISASSEMBLY ALLEN WRENCH  
(OR STANDARD 9/64" ALLEN WRENCH)

**Figure 11-3. Pump Head Assembly**

## 11.4 MAINTENANCE OF THE UV OPTICS MODULE AND THE CONDUCTIVITY FLOW CELL

The following two sections discuss the cleaning of the UV Optics module and the Conductivity flow cell and replacement of the lamp in the UV Optics module.

### 11.4.1 Cleaning the UV Optics Module and the Conductivity Flow Cell

Cleaning of the UV flow cell is indicated when the baseline is very noisy or unstable yet air bubbles have been removed from the flow cell. However, problems with UV detection during medium pressure chromatography are often solved by a few simple precautions. Air bubbles trapped in the flow cells (especially the “Z” cell) give rise to a “sawtooth” signal pattern that will never stabilize. We recommend always running with a backpressure regulator after the Optics module (if using low pressure columns, place the back pressure regulator between the pump outlet and inject valve). Equilibrate the column for at least 30 minutes to allow all bubbles to clear. Always degass buffers extensively before use.

Make sure all fittings are tight to prevent leaks. If liquid leaks down between the flow cell and the optics module case, the cell should be removed and dried with a stream of pure nitrogen (never use compressed air). The case should be dried using a warm air blower or left for several hours to completely dry.

Air bubbles trapped in the Gradient Pump give rise to erratic flow rates that are sometimes interpreted as problems with the UV optics module. Refer to the previous section.

If cleaning of the UV Optics module and the Conductivity flow cell is desired, note the following:

- **Filters:** The standard filter tray contains both a 280 nm and a 254 nm filter. To clean the filters, loosen the filter’s thumbscrew and lift out the filter holder. The filters should be cleaned only when necessary using a dry lens tissue. To remove greasy fingerprints, use dilute ethanol or isopropanol solutions.
- **UV Flow Cell:** To remove the flow cell, undo the flat bottom fittings at the top and bottom of the flow cell. Then loosen the thumbscrews and push out the flow cell from the bottom. The flow cell is keyed to mount in one way only, so that it cannot be incorrectly mounted. Avoid touching the quartz windows with fingers.

If the flow cell is visually dirty, it should be cleaned with one or more of the following solvents: dilute SDS, 1M HCl, 1M NaOH, ethanol, or acetone. Run the solvent through the flow cell using a syringe and leave for no more than 5 minutes. Rinse extensively with water and then blow dry using a gentle stream of pure nitrogen. **Never** dry with compressed air from a “house” line as this will contain microdroplets of oil that will coat the cell.

When the Optics module is not in use, disconnect the flow cell and use a syringe filled with distilled water to clean out traces of salts and protein.

Before storing the flow cell, inject a dilute solution (10% to 25%) of ethanol or isopropanol into the cell to prevent microbial growth. Use the plugs provided with the flow cell to seal the flow cell’s inlet and outlet lines.

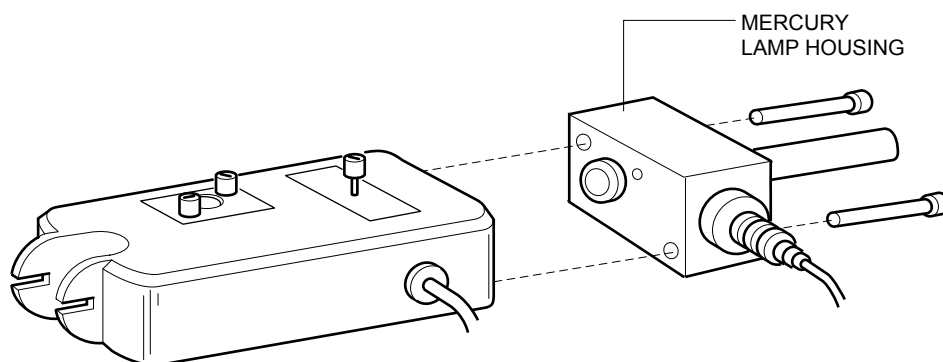
- The Conductivity cell requires little maintenance other than rinsing routinely with deionized water.

### 11.4.2 Replacing the Lamp in the UV Optics Module

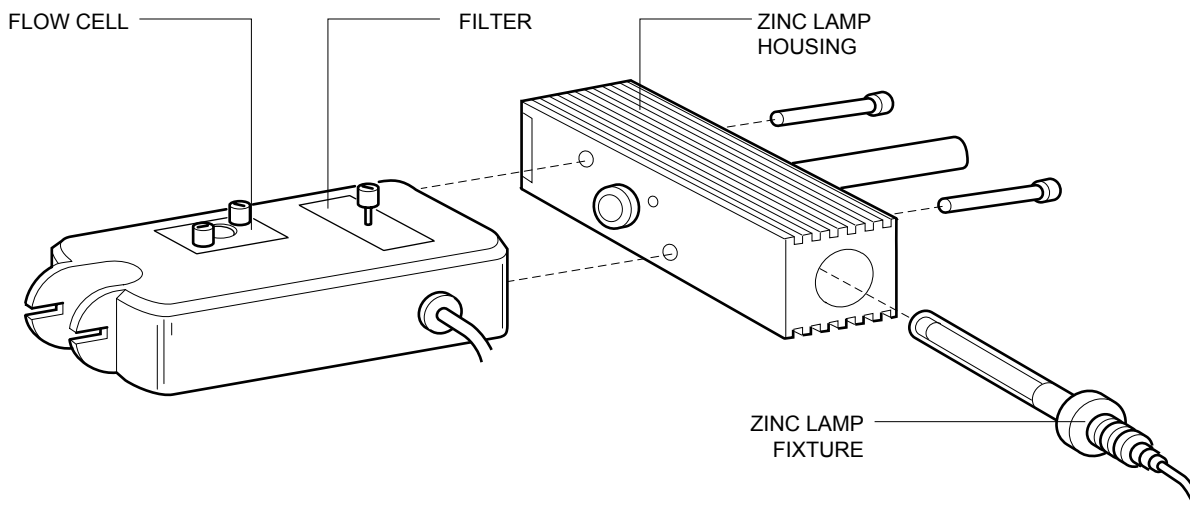
The following information covers the replacement procedures both for a new mercury lamp unit and a new zinc lamp unit. Note that the zinc lamp assembly has cooling fins and is larger than the mercury lamp assembly.

Indications that a lamp replacement is necessary include an unstable baseline and a decreased response to a standard concentration of a test chromophore. In this latter case, it is advisable to ensure that the flow cell is clean and that the correct wavelength filter is chosen before replacing the lamp. Refer to the previous section.

**Note:** Before performing the following procedure, make sure the BioLogic Duo-Flow Workstation is turned off. This will eliminate the risk of electric shock to you or damage to the Optics module during cable connection.



**Figure 11-4. Replacing a Mercury Lamp in a Model OM-II Optics Module**



**Figure 11-5. Replacing a Zinc Lamp in a Model OM-II Optics Module**

1. At the BioLogic Duo-Flow Controller go to the Manual screen and turn off the lamp. Turn off power to the Workstation and unplug the UV Lamp cable, which is plugged into the UV Lamp connector on the rear of the Workstation.
2. Without disconnecting the lamp housing from the UV bench, undo the two small Allen screws securing the lamp fixture and gently pull the lamp out of the housing.
3. Remove the lamp unit from its packing material.  
**WARNING:** Do not touch the glass part of the lamp. Oils from your fingers will degrade the lamp over a period of time.
4. Carefully insert the replacement lamp fixture. The replacement lamp can be installed only in one orientation due to a small guide which fits into the housing. Secure it using the Allen screws for that purpose. Note that extra screws are provided with the kit.

**Note:** Check that the O-ring seal at the Lamp Housing aperture is present and undamaged. If there is any indication of damage, or if it is missing, be sure to replace it using the O-ring supplied with the kit.

5. Reconnect the UV Lamp cable to the Workstation.
6. Turn on electrical power to the Workstation and at the BioLogic Duo-Flow Controller go to the Manual screen and turn on the lamp. Allow at least 30 minutes for the mercury lamp to warm up and at least several hours for the zinc lamp.

## 11.5 MX-1 MIXER

The Mixer may be disassembled for cleaning or to change the mixer capacity to accommodate the flow rate. Cleaning may be required if there is erratic gradient performance.

The Mixer consists of the mixer base and barrel, magnetic stir bar, screws, mixer top, and sealing O-rings.

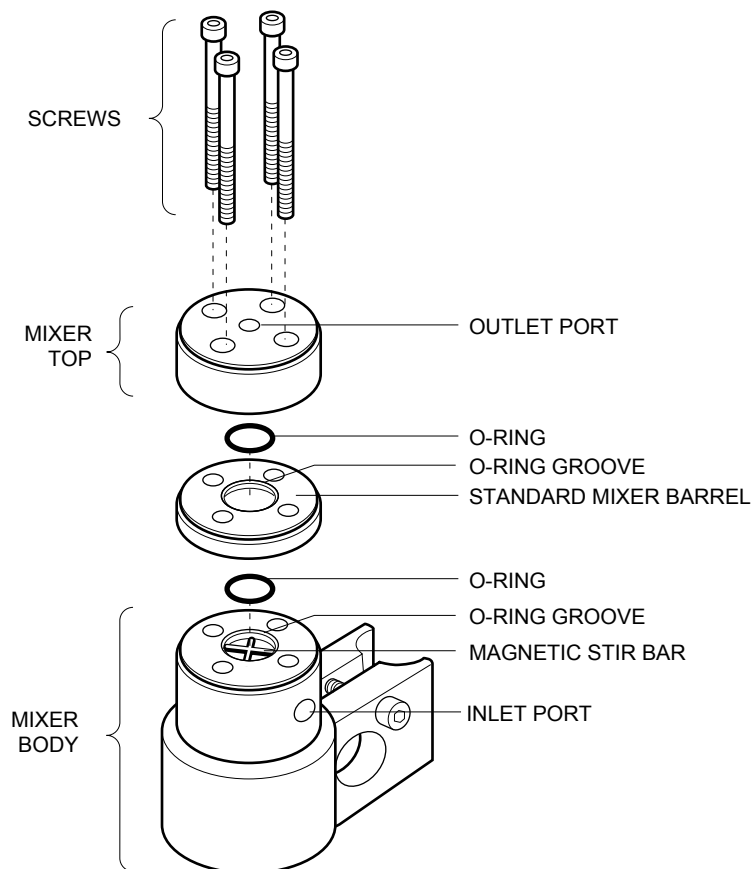
**Note:** If the system has been used, make sure that any hazardous material has been flushed from the system, the pumps are not running, and any residual pressure has been bled from the system. Drain fluid from the mixer, disconnect any plumbing connections, disconnect the cable from the workstation, and then remove the mixer from the system rack.

1. Use the hex key provided (or any standard 5/32" hex key) to remove the four assembly screws from the top of the Mixer.
2. Remove the Mixer Top and turn it upside down to remove the O-ring. If the O-ring does not easily dislodge, then use your fingers to remove it.  
Note the position of the O-rings, as shown in Figure 11-6. Remove the stir bar, if necessary.
3. To clean the mixer body, insert a 1/4-28 plug into one inlet and a 10 ml syringe into the other inlet port and flush the system with DI water. Reverse the location of the plug and syringe and repeat. To clean the mixer barrel or mixer barrel extension, the O-rings, and the magnetic stir bar, soak or sonicate in a bath containing a mild detergent. Replace damaged O-rings.

**Note:** The stir bar is magnetized. To remove it, turn the mixer base upside down and tap it on your hand. To re-insert the magnetic stir bar, place it on a tabletop and carefully place the mixer base over it.



4. Refer to Figure 11-6 for re-assembling the Mixer. Make sure that the magnetic stir bar lies flat and that the O-ring groove in either the Standard Mixer Barrel or the Mixer Barrel Extender faces up. Place an O-ring in each O-ring groove. If you are using only the Mixer Body (263 $\mu$ L), then only one O-ring is required. If either the Standard Mixer Barrel or the Mixer Barrel Extender is used, then two O-rings are required.



**Figure 11-6. Mixer Assembly**

## 11.6 VALVES

The following sections discuss the procedures for cleaning and repair of the SVT3-2, AVR7-3 and AVR9-8 valves.

### 11.6.1 SVT3-2 Diverter Valve

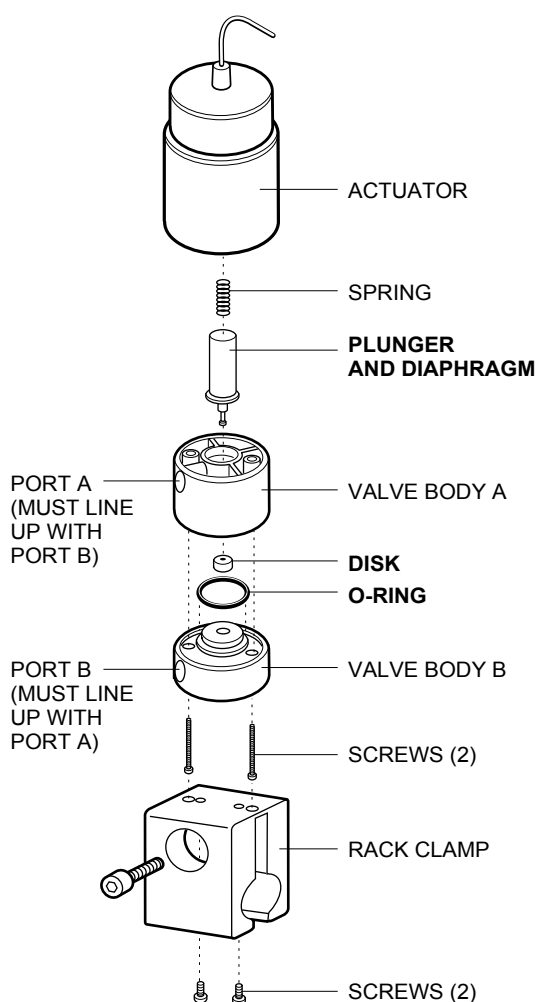
The SVT3-2 can be disassembled for cleaning or to replace parts which may become damaged over time. Indications of damage include valve leaks and valve switching problems. Items labeled in bold in Figure 11-7 are included in the SVT3-2 valve rebuild kit, catalog #760-0411. You will need a #1 Phillips (cross-head) screwdriver and a #0 Phillips (cross-head) screwdriver.

**Note:** These parts are rather small. Be sure you are working on a clean tabletop with room to lay out these parts and that your hands are clean.

**Safety Note:** Before disassembling the valve, be sure to flush any hazardous material from the valve and to disconnect it from the Workstation. Wear protective clothing, as appropriate.

To disassemble the valve, follow the procedure below:

1. Remove the large screw that attaches the clamp to the rack.
2. Use the #1 Phillips (cross-head) screwdriver to remove the two screws that attach the clamp to the valve. Set the clamp and screws aside.



**Figure 11-7. SVT3-2 Valve Assembly**

3. Use the #0 Phillips (cross-head) screwdriver to remove the two screws and washers that secure valve bodies A and B to the actuator. Set the actuator aside, taking care not to lose the spring.
4. Use your fingers to remove the plunger/diaphragm assembly from the valve body.
5. Remove the label. A new label is provided in the rebuild kit. Note that ports A and B are on one side; the Pump/Common port is on the other side of valve body A. When reassembled, the new label will not fit properly if ports A & B are not adjacent to each other.
6. Carefully separate the two valve bodies. Note the location of the O-ring.
7. Inspect the disassembled valve. Make sure there are no scratches or foreign material on the sealing surfaces of the two valve bodies. Clean the valve bodies, if necessary, by soaking or sonicating in a bath containing a mild detergent. Replace all required parts, if necessary.

To reassemble the valve:

1. Insert the plunger/diaphragm assembly into valve body A. Invert it and install the sealing disk onto the plunger tip. Make sure the disk inserts into the plunger tip.
2. Install the O-ring on valve body B. Replacing the O-ring is easily done with your finger.
3. Put the two valve bodies together so that the two ports are next to each other. The two valve bodies must lie flush against each other, with no space in between them. Place the two larger screws (removed in step 3 above) through the valve assembly. Set this aside.
4. Set the actuator with its open end facing up.
5. Place the spring in the opening at the center of the actuator so that it fits into the deepest part of the opening. This may take a couple of tries.
6. Place the valve body assembly onto the actuator (the orientation is not specific), then tighten the two screws.
7. Attach the clamp to the valve body with the remaining screws.
8. Apply the valve label. Make sure that the hole in the label matches with the Pump/Common port and does not overlap it.

### 11.6.2 AVR7-3 and AVR9-8 Valves

The AVR7-3 and AVR9-8 valves are disassembled and cleaned in the same way. The replaceable parts in the valve are the Rotor Seal and the Stator Face assembly; these items are included in the AVR7-3 rebuild kit (catalog #760-0401) and the AVR9-8 rebuild kit (catalog #760-0403). Indications of damage include valve leaks and valve switching problems.

**Safety Note:** Before disassembling the valve, be sure to flush any hazardous material from the valve and to disconnect it from the Workstation. Wear protective clothing, as appropriate.

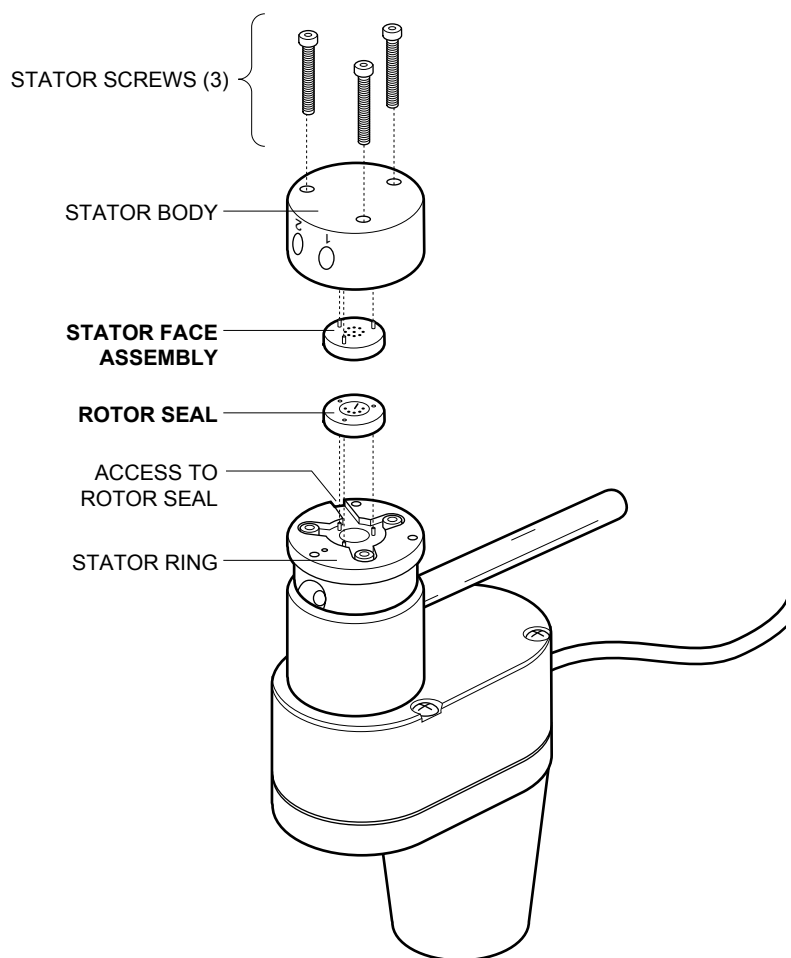
To disassemble the valve, follow the procedure below:

1. With the hex key that is provided, remove the three stator screws.
2. Lift the Stator Body and Stator Face assembly from the Stator Ring. (The Stator Face assembly usually stays with the Stator Body when it is lifted off.)

3. Remove the Stator Face Assembly with your fingers and set it aside.
4. Remove the Rotor Seal by placing a small flat blade screwdriver into the slot on the side of the Stator Ring. Gently pry up the Rotor Seal, which will pop off.
5. Inspect the components from the disassembled valve. Make sure there are no scratches or foreign material on the sealing surfaces of the valve. Clean as necessary. Soak or sonicate in a bath containing a mild detergent.

To reassemble the valve:

1. Place the new Rotor Seal (slot-side facing the Stator Body), onto the three seal pins of the Stator Ring. It will only fit one way. Press the Rotor Seal so that it fits firmly.
2. Place the new Stator Face Assembly onto the Stator Body so that the pins slip into the mating holes in the Stator Body. It will only fit one way.
3. Replace the Stator Body and Stator Face Assembly so that the Stator Ring enters the mating hole on the Stator Body.
4. Using the three screws, re-assemble the valve. Tighten each screw equally.



**Figure 11-8. AVR7-3 and AVR9-8 Valve Assembly**



## 12.0 TROUBLESHOOTING THE BIOLOGIC DUO-FLOW SYSTEM

The following section lists potential problems and some suggested solutions for the different instruments in the system.

### 12.1 TROUBLESHOOTING THE BIOLOGIC DUO-FLOW CONTROLLER AND SOFTWARE

Problem	Possible Cause	Solution
Software response is slow.	Database size may need to be reduced.	<ol style="list-style-type: none"> <li>1. Archive unnecessary methods and runs.               <ol style="list-style-type: none"> <li>a. Open the <b>Browser</b> window.</li> <li>b. Use <b>COPY OUT</b> to archive seldom used methods and runs. You may archive to a floppy disk, the BioLogic "C" drive, or any other drive selectable from the Drives drop down menu.</li> </ol> </li> <li>2. From the Browser &amp; Database Options window (available by selecting <b>Browser Settings</b> from the <b>Options</b> menu), make sure that the <b>Optimize on Startup</b> button is selected.</li> <li>3. Use the Windows 95 <b>Disk Defragmenter</b> utility. (Minimize the BioLogic Duo-Flow application, and then from Windows Start, select Programs, Accessories, System Tools, Disk Defragmenter. Follow the on-line instructions to defragment files on your disk.</li> </ol>
A method or run you expect is not displayed in the Browser.	There may be no apparent cause for this. Use the <b>Reset</b> button in the Browser toolbar.	<ol style="list-style-type: none"> <li>1. From the Browser toolbar, click on the <b>Reset</b> button, and then check the database tree for your method or run. <b>Note:</b> Using <b>Reset</b> collapses the database tree.</li> </ol>
While in offline mode, the offline window is not displayed.	The Windows® 95 minimize button may have been selected.	<ol style="list-style-type: none"> <li>1. Use any of the following three means of activating the offline window:               <ol style="list-style-type: none"> <li>a. Use the Windows drop-down menu.</li> <li>b. Use the Windows® 95 taskbar.</li> <li>c. Simultaneously hold down the Alt-Tab keys.</li> </ol> </li> <li>2. Click on the right mouse button and from the menu that is displayed, select <b>Restore</b>.</li> </ol>
Problems using Browser functions.	If you are using the right mouse button rather than the toolbar buttons, make sure you have first used the left mouse button to highlight the method or run name.	<ol style="list-style-type: none"> <li>1. Using the left mouse button, click on a method or run name.</li> <li>2. Using the right mouse button, select a function from the drop-down menu that is displayed.</li> </ol>

Problem	Possible Cause	Solution
Instrument face-plates (eg. pump, UV, fraction collector) “drop-off” the screen when in Manual mode.	A communication problem has occurred.	<ol style="list-style-type: none"> <li>1. Exit the BioLogic application and turn off the Controller. Turn off the Workstation.</li> <li>2. Make sure all Instrument Bus (phone jack style) connections are secure in all the instruments. Turn the Controller and Workstation back on.</li> <li>3. If the problem persists, contact Bio-Rad Technical Service.</li> </ol>
Controller monitor displays unusual colors or characters.	The Monitor or the Controller video card may be defective and need replacement.	<ol style="list-style-type: none"> <li>1. Exit the BioLogic application and restart the Controller.</li> <li>2. If the problem persists, contact Bio-Rad Technical Service.</li> </ol>
No sound from monitor speakers.	<p>Cables not connected properly.</p> <p>“Mute” switch is activated.</p>	<ol style="list-style-type: none"> <li>1. Consult the monitor instruction manual to ensure that the cables are correctly connected.</li> <li>2. Press the “mute” button, which is located on the front of the monitor to turn on the speakers.</li> </ol>
Controller displays “General Protection Fault” message or “locks up” during normal operation.		<ol style="list-style-type: none"> <li>1. Exit the BioLogic application and restart the Controller.</li> <li>2. If the problem persists, contact Bio-Rad Technical Service.</li> </ol>

## 12.2 TROUBLESHOOTING THE BIOLOGIC DUO-FLOW GRADIENT PUMP

Problem	Possible Cause	Solution
Pump is not delivering the correct flow rate.	The incorrect pump heads may be mounted, or the software Configuration utility may not have been run after the pump heads were changed.	1. Make sure the correct pump heads (labeled F-10 or F-40 on the lower-right corner of the center block) are mounted. Then run the pump head software Configuration utility.
	There may be problems with the fittings or tubing sizes. It is important to use the wide bore 1/8" OD tubing for the pump inlets and from all pre-pump buffer selection valves to the buffer containers.	2. Check pumps A & B and pre-pump valves. <ol style="list-style-type: none"> <li>Make sure the inlet lines into pumps A and B are tight.</li> <li>If pre-pump valves are used, e.g., for buffer selection, make sure all tubing connections are secure and any non-used ports are plugged.</li> </ol>
	The pump may not be receiving buffer.	3. Check that there is buffer flowing to the pump. <ol style="list-style-type: none"> <li>Ensure that the pump inlet lines are immersed in buffer.</li> <li>Ensure that the buffer bottles are positioned at or above the level of the Workstation.</li> </ol>
	There may be air bubbles trapped in the pump heads, causing erratic liquid delivery.  <b>Note:</b> Always degas buffers before use.	4. Re-prime the pumps and purge the lines. <ol style="list-style-type: none"> <li>Re-prime the pumps and ensure that the priming port is shut tight.</li> <li>Make sure the Inject Valve is set to Purge position and purge all lines.</li> </ol> Refer to the Maintenance section on how to prime the gradient pump and remove trapped air bubbles. To minimize the problem of air bubbles, degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum.
	Check valves are fouled and should be replaced.  The check valves should be replaced on a regular basis, depending on the usage and type of solution or sample pumped through the pump head. Refer to the Maintenance section for more information on how to replace a check valve.	5. Clean (or replace) the check valves (see section 11.3.3, Routine Maintenance of the Gradient Pump.) <ol style="list-style-type: none"> <li><b>Cleaning:</b> Sonicate check valves in a warm detergent solution, rinse with DI water, and re-install. <b>Replacing:</b> Replace badly clogged or damaged check valves.</li> <li>Recalibrate the pumps after cleaning/replacing check valves. Select <b>Calibrate Gradient Pump</b> from the <b>Utilities</b> drop-down menu.</li> </ol>



Problem	Possible Cause	Solution
Liquid is continuously leaking from the pump-head washout drain trough.	The pump's piston seals are worn or damaged.	<ol style="list-style-type: none"> <li>1. Replace the piston seals on both pump heads. Clean pistons and piston guides with water, followed by methanol as a precautionary maintenance step.</li> <li>2. Recalibrate the pumps after changing seals by selecting <b>Calibrate Gradient Pump</b> from the <b>Utilities</b> drop-down menu.</li> </ol>
Erratic or reduced flow rate when pumping onto columns which produce low back-pressures (eg., Econo-Pac® cartridges or small, agarose gel affinity columns).	Columns packed with soft gels or large particles typically produce very low back-pressures when run at low flow rates.	<ol style="list-style-type: none"> <li>1. Insert the back-pressure regulator between the pump and the injection valve.</li> </ol> <p><b>CAUTION: Low-pressure columns may burst if the backpressure device is placed after the column.</b></p>
Delivery of liquid is erratic and the pressure readout on the Controller status bar is fluctuating more than 20%.	Severe fluctuation in the back-pressure readout indicates an air bubble trapped in one or both of the pump heads.  <b>Note:</b> Always degas buffers before use.	<ol style="list-style-type: none"> <li>1. Put the AVR7-3 valve into the purge position and purge the system by pressing the Purge buttons on the front of the Workstation panel.</li> <li>2. Stop or Pause the pumps and re-prime. Loosen the top fitting on the pump head and force any trapped air out of the pump head by squirting buffer through the priming port using a syringe. Refer to the maintenance section on how to prime the gradient pump and remove trapped air bubbles by using methanol. Always use degassed buffers.</li> <li>3. To minimize this problem, use degassed buffers and solutions. Degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum.</li> </ol>
	The pump's check valves may need cleaning or replacement.	<ol style="list-style-type: none"> <li>4. Clean (or replace) the check valves (see section 11.3.3, Routine Maintenance of the Gradient Pump.)               <ol style="list-style-type: none"> <li>a. <b>Cleaning:</b> Sonicate check valves in a warm detergent solution, rinse with DI water, and re-install.</li> <li><b>Replacing:</b> Replace badly clogged or damaged check valves.</li> <li>b. Recalibrate the pumps after cleaning/replacing check valves. Select <b>Calibrate Gradient Pump</b> from the <b>Utilities</b> drop-down menu.</li> </ol> </li> </ol>

Problem	Possible Cause	Solution
Loud or unusual noise coming from the front of the workstation.	Sticky pistons.	Follow the maintenance procedure to carefully remove and disassemble the pumpheads. Wash the pistons thoroughly with water to remove debris or salt crystals. If the piston seals have many hours of use, they should be replaced following the information in the maintenance section. After re-assembly of the pump heads, pump 100 ml of 100% methanol (MeOH) through each pumphead at 5 ml/min with the 40-psi backpressure device in place.
	Dirty Pistons.	2. The pistons should be rinsed on a nightly basis. This may easily be done automatically by programming a water rinse step at the end of each protocol. This may also be done manually by washing the pistons with the syringe provided in the Fittings Kit.
The back-pressure displayed on the Controller status bar drops to zero or is much lower than expected.	Flow rate may have been changed.	1. Confirm that the flow rate setting has not been changed.
	There may be a tubing or fitting leak.	2. Re-inspect all tubing connections, especially at the pump inlet.
	Pump seals may need replacement.	3. Replace pump seals. A build-up of crystallized buffer salts on the rear of the pump head is a clear sign for seal replacement. (see 11.3.3, Routine Maintenance of the Gradient Pump.)
	Check valves may need cleaning or replacement.	4. Clean or replace check valves. (see section 11.3.3, Routine Maintenance of the Gradient Pump.)
No pressure (psi) reading on Controller status bar or psi value always reads zero.	Pump may not be running, or inlet lines are not primed, or there is a problem in the buffer flow.	1. Check that the pumps are running, and that the inlet lines are primed and not pulled out of the buffer reservoirs. Check for leaking fittings. 2. Check that the buffer flow is going to the column and not to waste via the injection valve purge position.
	The “zero pump pressure” calibration routine may have been used while the system was under pressure.	3. If this last cause is suspected, stop the pump and undo the line from the pump to the valve such that the pressure transducer is definitely at zero psi. Select <b>Calibrate Gradient Pump</b> from the <b>Utilities</b> drop-down menu and perform a <b>Zero psi</b> routine.

Problem	Possible Cause	Solution
Incorrect back-pressure readings for a given column.	Flow rate may have been changed.	1. Check that the pump is set to deliver the correct flow rate.
	Column may need cleaning along with a frit replacement.	2. Consult the column's instruction manual for cleaning procedure. Alternatively, the system tubing may have an obstruction, so inspect the tubing path.
	The pressure transducer calibration may be incorrect.	3. Stop the pump, momentarily loosen the pump outlet fitting to release pressure. Select <b>Calibrate Gradient Pump</b> from the <b>Utilities</b> drop-down menu and perform a " <b>Zero psi</b> " routine.
High back-pressure is shutting down the pumps until the pressure drops.	<p>Maximum pressure for the pumps is: F-10: 3500 psi F-40: 1000 psi.</p> <p>The pressure limit settings in the Manual screen (see Figure 7-2) have been set too low.</p> <p>Pressure increases are due to a build-up of particulate matter in the system, causing a resistance to flow. An increase in flow rate also increases the pressure, so be sure to check this setting.</p>	<p>1. Remove the column and run the pumps to determine if the high back-pressure is due to the column or not. If the column is suspect, the column may need cleaning along with a frit replacement. Consult the column's instruction manual for cleaning procedure.</p> <p>2. Alternatively, the system tubing may have an obstruction, so inspect the tubing path. To isolate the blockage, start by loosening connection fittings at the detector and work backwards towards the column and pump head.</p> <p>3. Prevent or minimize high back-pressure problems by filtering buffers and samples and by changing the pump seals before they deteriorate completely.</p>

## 12.3 TROUBLE SHOOTING THE UV OPTICS MODULE AND UV TRACE

Problem	Possible Cause	Solution
<p>UV baseline is unstable or noisy.</p> <p>UV baseline shows a reproducible “zig-zag” or “saw-tooth” trace.</p>	<p>Air bubbles trapped in the analytical 5-mm Z- cell are the most common cause of noisy UV baselines.</p> <p><b>Note:</b> Always degas buffers before use.</p>	<ol style="list-style-type: none"> <li>1. Degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum.</li> <li>2. Plumb the Z-cell with the column outlet connected to the bottom of the flow cell. This will force air bubbles to rise to the top of the flow cell and dissipate.</li> <li>3. Use of the 40-psi back-pressure regulator supplied with the BioLogic Duo-Flow system will remove most air bubbles. Place it directly after the conductivity flow cell and run buffer for several minutes.</li> </ol> <p>If the column being used cannot be run with the back-pressure regulator and air bubbles are a continuing problem (for example, during some affinity chromatography steps), use the 2mm path length cuvette cell. This cell is approximately 2.5x less sensitive than the Z-cell and is appropriate in those cases where the concentration of protein sample is high enough to be seen on the chromatogram.</p>
Excessive pump pulsations exhibited as “regular noise” on the baseline.	Air bubbles trapped in the pump heads can produce exaggerated pulsations which appear as a noisy baseline.	<ol style="list-style-type: none"> <li>4. Purge the pump heads to remove the bubble. Refer to section 11.3.1, Priming the Gradient Pump and Removing Trapped Air Bubbles.</li> </ol>
Baseline noise continues when pumps are turned off.	Noise spikes can also be caused by external environmental influences.	<ol style="list-style-type: none"> <li>1. If the spikes occur at regular intervals (for example, once every 20-30 sec), check for the presence of heating baths, drying ovens, or other heating devices on the same electrical circuit or in close proximity to the BioLogic Duo-Flow system. Turn off these devices to see if the problem goes away.</li> </ol>

Problem	Possible Cause	Solution
UV trace will not zero.	If no air bubbles are present in the flow cell, check that the cell itself is clean both internally and externally.	<ol style="list-style-type: none"> <li>1. Clean the interior of the cell by passing 1M NaOH, water, methanol and water through the cell. Dry the interior of the cell with a stream of high purity nitrogen gas (never use "house" compressed air as it may contain oil droplets).</li> <li>2. Check for leakage of liquid from the top of the optics unit onto the outside quartz windows of the flow cell. Clean the windows with a soft, non-abrasive damp cloth.</li> <li>3. Condensation may occur on the exterior windows of the flow cell when moving the optics unit from the lab to a cold room and vice versa. Always allow an equilibration period to compensate for such temperature effects.</li> </ol>
Drifting baseline.	Non-homogeneous eluant perhaps due to poor mixing or flow rate variation.	1. Check flow rates of both pumps; recalibrate if needed by selecting <b>Calibrate Gradient Pump</b> from the <b>Utilities</b> drop-down menu.
	Slow column equilibration.	2. Certain ion-exchangers are slow to re-equilibrate especially if just sanitized. Allow a longer equilibration period.
	If drift is due to a higher absorbance, suspect that UV absorbing material may be leaching from the column.	3. To confirm this, remove the column and run DI water or an aqueous non-absorbing buffer. If the baseline is stable, then the column is suspect. Consult the column's user manual for cleaning procedures.
Negative peaks.	The sample is applied to the column in a different buffer from that used to equilibrate and elute the column. Refractive index changes may be responsible for the negative peaks.	1. Apply the sample in the same buffer used to equilibrate the column.
	The elution buffer may have a higher UV absorbance than the sample components.	2. Check the elution buffer's UV absorbance.

Problem	Possible Cause	Solution
False or “ghost” peaks.	<p>The injection valve, injection port and loop were not rinsed out between sample injections.</p> <p>Strongly-retained sample components may still be eluting from a previous run.</p>	<ol style="list-style-type: none"> <li>1. It is a good idea to allow a larger volume of injection buffer to pass through the inject valve and loop. For example, if the sample loop contains 100 µl, then program a load/injection volume of 200 µl to completely flush the valve and loop.</li> <li>2. Always clean large volume dynamic loops, such as the Bio-Rad DynaLoop, between use to avoid cross contamination.</li> <li>3. Use stringent elution conditions or consult the column’s user manual for an appropriate wash step to remove such components between runs.</li> </ol>

#### 12.4 TROUBLE SHOOTING THE CONDUCTIVITY FLOW CELL AND TRACE

Problem	Possible Cause	Solution
No values for buffer conductivity are displayed on the Controller status bar.	There may be a loose or incorrect cable connection.	1. Make sure the cable from the conductivity flow cell is plugged into the correct connector on the rear of the Workstation and that the pins are not bent.
	Conductivity Monitor may need calibration.	2. Use the <b>Conductivity Flow Cell Constant Calibration</b> function from the Utilities pull-down menu. Either input the constant value which is shown on the tag attached to the flow cell or calibrate the flow cell using 0.5M NaCl. Note that the relationship between the conductivity reading and salt concentration is not linear. The curve will flatten out at higher salt concentrations.
Conductivity trace does not follow exactly the theoretical gradient trace %B.		1. The Conductivity flow cell is typically placed after the UV flow cell. The Conductivity trace will be offset from the theoretical gradient based upon the column and system volume.
Distorted conductivity trace.	Mixer capacity is incorrect	1. Refer to section 2.3, Mixer, for discussion of mixer capacities.

## 12.5 TROUBLE SHOOTING THE MODEL 2128 FRACTION COLLECTOR

Problem	Possible Cause	Solution
The drops are missing the tubes.	The rack being used does not correspond to the rack selected by the BioLogic Duo-Flow software.	1. Ensure that the correct Rack # (ie., #1 to 5) has been entered in the Method Protocol and matches the actual rack being used.
	The fraction collector may need a drop-head alignment.	<p>2. Model 2128 Fraction Collectors with firmware v. 1.03 or later have a drop-head alignment feature. To align the drop-head, the fraction collector must be in “stand-alone” mode (i.e., not in BioLogic mode). Follow the procedure below:</p> <ol style="list-style-type: none"> <li>When you first turn on the fraction collector, note the firmware version as it is briefly displayed. The firmware must be v. 1.03 or greater.</li> <li>From the startup display, press the <b>Mode</b> softkey.</li> <li>Simultaneously press the “0” and “.” hardkeys.</li> <li>From the menu, press the <b>Calib</b> softkey.</li> <li>From the calibration screen, press the <b>D-Arm</b> softkey.</li> <li>Respond to the message, “Initialize Drop Arm?” by pressing the <b>Yes</b> softkey.</li> <li>Respond to the message, “Select Item to be Repositioned,” by pressing the <b>Rack</b> softkey.</li> <li>To align the drop-head over a specific tube, press the <b>Locate</b> softkey, followed by the <b>Adjust</b> softkey.</li> <li>Adjust the position of the drop-head over the tube using the four “arrow” hardkeys. It is helpful to have liquid flowing during this procedure.</li> <li>It is best to check the alignment at two positions. For example, for rack #1, check the drophead alignment over tubes 1 and 8. Use the <b>Adv</b> softkey to select the next tube.</li> <li>When you are done, press the <b>Done</b> softkey, followed by the <b>Exit</b> softkey and the <b>Yes</b> softkey to save the adjustment. Press the <b>Exit</b> softkey twice to return to the startup screen. The fraction collector must be in <b>Std</b> mode before it can be returned to BioLogic system control.</li> </ol> <p>3. Make sure that the tubing is not being snagged and restricting the movement of the drophead.</p>





## APPENDIX A. SPECIFICATIONS

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### System Power Requirements: BioLogic Duo-Flow Controller and Workstation

- 100 V ~ 5.3 A
- 230 V ~ 3.5 A
- 50 - 60 Hz

### BioLogic Duo-Flow Controller

- Pentium®/300 MHz, 6 GB hard drive, 32 Mb RAM
- 3.5" floppy drive; CD-ROM drive;
- Super VGA color monitor
- PC-compatible mouse and PS/2 keyboard
- Windows® 95 operating system
- BioLogic Duo-Flow software, providing single point control of all chromatographic parameters including fraction collection and editing a Method during a run
- Optional chromatogram integration software
- Networkable for printer and file access

### Gradient pump

- Dual piston, positive displacement solvent pumps, with interchangeable solvent delivery pumpheads (F-10 and F-40) made of bio-compatible PEEK
- F-10 pumpheads: 3,500 psi (233 bar, 23 MPa) from 0.01 to 10.0 ml/min in 0.01 ml/min increments  
Flow accuracy: 0.01 to 10.0 ml/min: 2%  
Flow reproducibility: 0.01 rsd
- F-40 pumpheads: 1,000 psi (66 bar, 6.6 MPa) from 0.5 to 40.0 ml/min in 0.01 ml/min increments  
Flow accuracy: 0.5 to 40.0 ml/min: 2%
- Compositional accuracy:  $\pm 1.0\%$
- High pressure dynamic mixing; mixer volume without barrel: 263  $\mu$ l, mixer with standard mixer barrel: 750  $\mu$ l; mixer with mixer barrel extender: 2.0 ml
- Built-in pressure transducer
- Gradient by software control: 1% increments

### General

- System fittings: Flangeless 1/4-28 flat-bottom fittings, fingertight nuts and ferrules
- System Tubing for flow rates up to 15 ml/min: 1/16" (1.6 mm) OD, 0.020" (0.51 mm) ID orange PEEK tubing. The approximate volume of 1 cm of tubing is 2  $\mu$ l.  
System Tubing for flow rates of 15 ml/min and greater: 1/16" (1.6 mm) OD, 0.030" (0.76 mm) ID green PEEK tubing. The approximate volume of 1 cm of tubing is 4.5  $\mu$ l.  
Pump Inlet Tubing: 1/8" (3.2 mm) OD, 0.062" (1.6 mm) ID PTFE tubing
- Wetted materials: 100% bio-compatible
- Solvent compatibility: 1.0 M NaOH, HCl and organic acids, 1.0 M Sulfuric acid, 7M Urea and Guanidine HCl, 100% Ethanol, IPA and Acetonitrile, 0.1% TFA, 1% detergents (incl. SDS, Triton X-100)
- Sound level, Workstation: <70 dBa
- Dimensions, Workstation (approx.): ~35 x 41 x 18 cm (W x D x H); height with 3-tray rack: 76 cm
- Weight (Controller, Workstation, monitor, rack, inject valve and detection modules): 34.5 kg (76 lbs) (approximate)

**Detection**

- Fixed wavelength, mercury lamp with 280/254 nm filter, auto zero; 0.0001 - 2.0 AUFS detection range
- Analytical (5 mm pathlength, 16 µl total volume) flow cell
- Preparative (2 mm pathlength 30 µl volume) flow cell
- Optional 214 nm detection kit (includes zinc lamp, housing, and 214 nm filter)
- Optional wavelength filters include: 214, 313, 365, 405, 436, and 546 nm
- Conductivity detection (500 mS/cm; 6 µl flow cell; accuracy  $\pm$  2% full scale)
- Optional flow-through pH monitor (includes flow cell, pH probe, and SIM-HR)

**Sample Loading**

- Fixed sample loops, 100 µl to 5.0 ml; automated injection valve included
- Large volume loading through the Gradient Pump. Optional inlet select valves (SV5-4, AVR9-8) for sanitation and multiple sample use.
- Variable volume sample loading through the DynaLoop sliding-piston sample loop.
- Software control of an auxiliary pump for multiple sample loop fills and large volume dynamic loop filling.

**Valve Control**

- Up to three low-pressure, automated solenoid valves (SV5-4 and SVT3-2) for stream selection.
- Up to three high-pressure, automated valves (AVR7-3 and AVR9-8) for sample inject, column switching and stream selection.

**Fraction Collection**

- Model 2128 Fraction Collector (collection by Collect All, Threshold, Collection Windows, Collection Windows + Threshold)
- Model 2110 Fraction Collector (collection by Collect All; when used with optional SVT3-2 Diverter valve, it also offers collection by Threshold, Collection Windows, Collection Windows + Threshold)

**Chart Recorder Control**

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

**Signal Import Module**

- Up to two Signal Import Modules for importing analog signals from peripheral chromatography detectors

**General**

- Working Dimensions (without collector and recorder): ~68 x 55 x 81 cm (W x D x H), depending upon configuration.
- Weight (Controller, Workstation, Monitor, Rack, Injection valve and Detection modules): 34.5 kg (76 lbs.)

## APPENDIX B. PRESSURE CONVERSION TABLE

Use this table to convert pressure units for the pump.

**Table B-1**  
**Pressure Conversion**

Multiply the units in the left column by the conversion factors listed.

Pressure	PSI	atm	Kg/cm <sup>2</sup>	Torr	kPa	Bar	Inches Hg
<b>PSI</b>	1	0.068	0.0703	51.713	6.8948	0.06895	2.0359
<b>atm</b>	14.696	1	1.0332	760	101.32	1.0133	29.921
<b>Kg/cm<sup>2</sup></b>	14.223	0.9678	1	735.56	98.06	0.9806	28.958
<b>Torr</b>	0.0193	0.00132	0.00136	1	0.1330	0.00133	0.0394
<b>kPa</b>	0.1450	0.00987	0.0102	7.52	1	0.0100	0.2962
<b>Bar</b>	14.5038	0.9869	1.0197	751.88	100	1	29.5300
<b>Inches Hg</b>	0.4912	0.0334	0.0345	25.400	3.376	0.03376	1

Example 1: Convert 521 psi to Kg/cm<sup>2</sup>  
(521 x 0.0703) or (521 psi = 36.63 Kg/cm<sup>2</sup>)

Example 2: Convert 60 Bar to psi  
(60 x 14.5038) or (60 Bar = 870 psi)

Example 3: Convert 42.70 Bar to Kg/cm<sup>2</sup>  
(42.70 x 1.0197) = (42.70 Bar = 43.54 Kg/cm<sup>2</sup>)

## APPENDIX C. WARRANTY STATEMENT

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Bio-Rad Laboratories warrants that every instrument it sells will be free from defects in materials and workmanship when it leaves the factory and that, if such defects appear within the first year following purchase, the defective part(s) will be replaced or the entire unit will be replaced, at Bio-Rad's option, free of any charges to the buyer other than expenses incurred in returning the unit to the factory. Bio-Rad's obligation under this warranty is specifically limited to the aforementioned replacement or repairs. However, the following defects are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or their authorized agent.
3. Use with fittings or other spare parts not specified by Bio-Rad Laboratories.
4. Damage caused by deliberate or accidental misuse.
5. Damage caused by disaster.
6. Damage due to use of improper solvent or sample.
7. Tubing, fittings, lamps, pistons, check valves, piston seals, and other consumables.

"The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties, of merchantability, fitness for a particular purpose or otherwise, expressed or implied in fact or by law, and state Bio-Rad's entire and exclusive liability and Buyer's exclusive remedy for any claims or damages in connection with the furnishing of goods or parts, their design, suitability for use, installation or operation, Bio-Rad will in no event be liable for any special, incidental or consequential damages whatsoever, and Bio-Rad's liability under no circumstances will exceed the contract price for the goods for which liability is claimed."

Under this warranty Bio-Rad's obligation with respect to transportation expenses is limited to the cost of shipping the repaired or replacement instrument to the buyer, provided that such repair or replacement comes within the terms of this warranty.

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

## APPENDIX D ORDERING INFORMATION

760-0101	BioLogic DuoFlow Controller
760-0150	BioLogic DuoFlow Workstation
760-0151	Priming Ports, 2
760-0180	F-40 Pump Upgrade Kit. Includes two F-40 pump center blocks, four F-40 piston seals, four piston assemblies, four pump O-rings, one mixer barrel extender, two mixer O-rings, a 2mm UV flow cell, PEEK tubing 0.030" (0.76 mm) ID, and necessary tools.
760-0164	F-10 Pump Maintenance Kit, services one 10-ml pump. Kit includes 2 piston seals, 4 check valves, seal removal tool, 2 O-rings.
760-0161	F-10 Piston Seals (2) and seal tool. Two piston seals required to service one pump.
760-0184	F-40 Pump Maintenance Kit, services one 40-ml pump. Kit includes 2 piston seals, 2 check valves, 2 pistons.
760-0172	F-40 Piston Seals (2) and seal tool. Two piston seals required to service one pump.
750-0162	Check Valve (1). Four check valves required to service one pump; for F-10 and F-40 pumps.

### Mixers

760-0170	MX-1 Mixer, Includes Mixer Body (263µL) and Standard Mixer Barrel (for total volume of 750µL)
760-0171	Mixer Barrel Extender (for total volume of 2mL)
100-1627	Mixer Service Kit
920-8529	Standard Mixer Barrel (1) (for total volume of 750µL)

### Detectors

750-0200	Detector Kit. Includes UV Detector with 254/280nm filter, 5mm flow cell, conductivity flowcell, and back-pressure device.
750-0214	214 nm Conversion Kit (Zn lamp, housing, 214 nm filter) only for Model OM-II Optics Modules beginning with serial number 362 BR.
750-0215	214 nm Conversion Kit (Zn lamp, housing, 214 nm filter) only for Model OM-IO Optics Modules, beginning with serial number 345 BR.
750-0210	UV Flow Cell, 2 mm path length
750-0212	UV Flow Cell, 5 mm path length
750-0216	Hg Lamp, replacement
750-0217	Zn Lamp (214 nm), replacement
750-0220	Filter, 280 + 254 nm
750-0221	Filter, 214 nm
750-0222	Filter, 313 nm
750-0223	Filter, 365 nm
750-0224	Filter, 405 nm
750-0225	Filter, 436 nm
750-0226	Filter, 546 nm
750-0240	Conductivity Flow Cell, replacement
750-0230	Back-pressure device
750-0505	BioLogic pH monitor (1 SIM-HR, 1 pH probe, 1 flow cell)
750-0502	Signal Import Module-HR
750-0503	pH electrode
750-0504	pH flow cell
750-0111	EZ Logic Integration Software Package

**BioLogic Valves**

760-0406	Automated Injection Valve, AVR7-3
760-0401	AVR 7-3 Valve Rebuild kit
750-0471	Sample Injection Port
760-0408	Automated Stream Select Valve, AVR9-8
760-0403	AVR 9-8 Valve Rebuild kit
760-0410	Diverter Valve, SVT3-2
760-0411	SVT 3-2 Valve Rebuild kit
750-0415	Select Valve, SV5-4
125-0224	Inject Needle, 22-gauge blunt

**750-0251 BioLogic Rack**

750-0268	Rack expansion kit (2 trays, 2 vert. bars, 16 sleeves)
750-0260	Column clamp set
750-0261	Rack tray; includes 8 sleeves, 1 drain/plug)
750-0262	Vertical bar, long (2)
750-0263	Vertical bar, short (2)
750-0264	Horizontal bar kit (2 tie bars, 4 bar clamps)
750-0265	Bar clamps (5)
750-0266	Cable Manager clips (4)
750-0269	System wrench set

**DuoFlow Sample Injection Loops**

750-0490	Small Volume Sample Loop Kit (100, 250, and 500µL loops). Nominal pressure limit of 4000 psi.
750-0491	Large Volume Sample Loop Kit (1, 2, and 5mL loops). Nominal pressure limit of 3000 psi.
750-0492	100 µl injection loop. Nominal pressure limit of 4000 psi.
750-0493	250 µl injection loop. Nominal pressure limit of 4000 psi.
750-0494	500 µl injection loop. Nominal pressure limit of 4000 psi.
750-0495	1 ml injection loop. Nominal pressure limit of 3000 psi.
750-0496	2 ml injection loop. Nominal pressure limit of 3000 psi.
750-0497	5 ml injection loop. Nominal pressure limit of 3000 psi.
750-0451	DynaLoop 25, for 25mL samples
750-0452	DynaLoop 90, for 90mL samples

**BioLogic DuoFlow Fittings**

760-0550	BioLogic DuoFlow Fittings Kit
750-0560	BioLogic Fittings Tool
760-0553	1/8" (3.2 mm) Pre-pump Fittings (includes nut, ferrule, and lock ring), 10
760-0554	1/16" (1.6 mm) Post-pump Fittings (includes nut, ferrule and lock ring), 10
750-0570	1/8" (3.2 mm) Nut, 5
750-0571	1/8" (3.2 mm) ferrule and lock ring, 5
750-0569	1/16" (1.6 mm) Nut, 10
750-0556	1/16" (1.6 mm) ferrule and lock ring, 10
750-0703	Inline Filter Kit, 1 inline filter, 2 replacement Ultra High Molecular Weight PolyEthylene (UHMWPE) filter frits
750-0704	Filter frits, replacement, 5
750-0566	Bottle Cap Kit, (includes 2 bottle caps, 2 cap plugs)
750-0559	Caps, 5 pk
750-0561	1/4-28 to M6 (FPLC column adaptor), 2
750-0562	1/4-28 to 1/4-28 union, 5
750-0563	1/4-28 plug, 5
750-0564	HPLC Column to BioLogic System Fittings Kit, 1 set
750-0565	Econo-Column to BioLogic System Fittings Kit

- 732-0113 Econo-Pac® Cartridge to BioLogic System Fittings Kit, 1 set  
750-0471 Econo-Column® to BioLogic System Fittings Kit, 1 set

### **Tubing**

- 750-0603 PTFE tubing, 1/8" (3.2 mm) OD x 0.062" (1.6 mm) ID x 15 feet (4.6 m), for pre-pump connections  
760-0604 PEEK tubing, 1/16" (1.6 mm) OD x 0.020" (0.51 mm) ID x 30 feet (9.2 m), for flow rates less than or equal to 10mL/min  
760-0605 PEEK tubing, 1/16" (1.6 mm) OD x 0.030" (0.76 mm) ID x 30 feet (9.2 m), for flow rates greater than 10mL/min

### **Fraction Collectors**

- 731-8123 Model 2128 Fraction Collector, 110 V  
731-8124 Model 2128 Fraction Collector, 220 V  
731-8238 Model 2128 Diverter Valve  
  
731-8122 Model 2110 Fraction Collector, 110 V  
731-8120 Model 2110 Fraction Collector, 220 V  
760-0410 Diverter Valve, SVT3-2  
  
750-0135 Starter Kit  
720-0001 UNO Q1 column  
760-0001 BioLogic DuoFlow Instruction Manual

### **Chart Recorder**

- 731-8250 Model 1327 Chart Recorder, with USA, Canada, Japan, Mexico, Taiwan, and Latin America power adaptor  
731-8253 Model 1327 Chart Recorder, with UK, Commonwealth power adaptor  
731-8254 Model 1327 Chart Recorder, with Australia, New Zealand power adaptor  
731-8255 Model 1327 Chart Recorder, with European power adaptor

### **Cables**

- 750-0650 System Cable 17 (bus cable), 4 feet (1.2 m)  
750-0651 System Cable 18 (bus cable), 12 feet (3.7 m)  
750-0652 System Cable 19 (bus cable), 30 feet (9.2 m)  
750-0655 System Cable 21 (bus cable), 100 feet (30 m)  
731-8262 System Cable 2 (mini-DIN to Model 1327 DIN)  
731-8264 System Cable 4 (mini-DIN to rec banana)  
731-8265 System Cable 5 (Workstation AUX bare wires to DB-9)  
731-8267 System Cable 7 (mini-DIN to bare wires)  
750-0653 System Cable 20 (mini-DIN to DIN)

## **APPENDIX E    COLUMN INFORMATION**

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The following contain information about the different columns available from Bio-Rad for use with the BioLogic Duo-Flow system.





**APPENDIX F    ISO 9001 CERTIFICATE**

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The following page shows the ISO 9001 Certificate of Registration for the BioLogic Duo-Flow system.



NATIONAL QUALITY ASSURANCE

# *Certificate of Registration*

This is to certify that the Quality Management System of

**BIO-RAD LIFE SCIENCES GROUP**  
**2000 ALFRED NOBEL DRIVE, HERCULES, CA 94547**  
(INCLUDED FACILITY AT: 85A MARCUS DRIVE  
PO BOX 1229, MELVILLE, L.I., NY. 11747)

applicable to

**DESIGN, DEVELOPMENT, AND MANUFACTURE OF  
CHEMICALS, AND ANALYTICAL INSTRUMENTS**

has been assessed and registered by  
National Quality Assurance Limited against the provisions of

**BS EN ISO 9001 : 1994**

This registration is subject to the company maintaining a quality management system,  
to the above standard, which will be monitored by NQA.

**The Seal of National Quality Assurance Limited  
was hereto affixed in the presence of:**

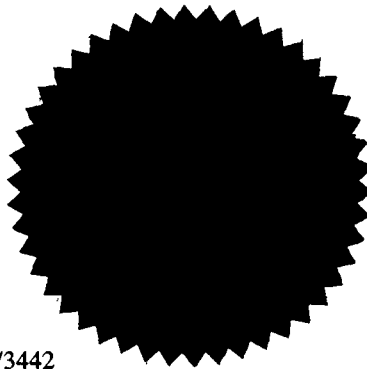
Managing Director



Certificate No: 5996

Date: 26 January 1995

Standard Industrial Classification Code 2567/3442



National Quality Assurance Ltd is registered in England. Registration No 2269505 Registered Office Vintage House 37 Albert Embankment, London SE1 7UJ



**Bio-Rad  
Laboratories**

*Life Science  
Group*

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**Also in:** **Australia** Ph. 02 9914 2800, Fx. 02 9914 2889 **Austria** Ph. (01) 877 89 01, Fx. (01) 876 56 29 **Belgium** Ph. 09-385 55 11, Fx. 09-385 65 54  
**Brazil** Ph. 55 21 507 6191 **Canada** Ph. (905) 712-2771, Fx. (905) 712-2990 **China** Ph. 86-10-8201-1366/68, Fx. 86-10-8201-1367  
**Denmark** Ph. 45 44 52-1000, Fx. 45 4452 1001 **Finland** Ph. 358 (0)9 804 2200, Fx. 358 (0)9 804 1100 **France** Ph. 01 47 95 69 65, Fx. 01 47 41 9133  
**Germany** Ph. 089 318 84-177, Fx. 089 318 84-123 **Hong Kong** Ph. 852-2789-3300, Fx. 852-2789-1257 **India** Ph. (91-124)-6398112/113/114, Fx. (91-124)-6398115  
**Israel** Ph. 03 951 4124, Fx. 03 951 4129 **Italy** Ph. 34 91 590 5200, Fx. 34 91 590 5211 **Japan** Ph. 03-5811-6270, Fx. 03-5811-6272  
**Korea** Ph. 82-2-3473-4460, Fx. 82-2-3472-7003 **Latin America** Ph. 305-894-5950, Fx. 305-894-5960 **Mexico** Ph. 52 5 534 2552 to 54, Fx. 52 5 524 5971  
**The Netherlands** Ph. 0318-540666, Fx. 0318-542216 **New Zealand** Ph. 64-9-4152280, Fx. 64-9-443 3097 **Norway** Ph. 47-23-38-41-30, Fx. 47-23-38-41-39  
**Russia** Ph. 7 095 979 98 00, Fx. 7 095 979 98 56 **Singapore** Ph. 65-2729877, Fx. 65-2734835 **Spain** Ph. 34-91-590-5200, Fx. 34-91-590-5211  
**Sweden** Ph. 46 (0)8-55 51 27 00, Fx. 46 (0)8-55 51 27 80 **Switzerland** Ph. 061-717-9555, Fx. 061-717-9550 **United Kingdom** Ph. 0800-181134, Fx. 01442-259118