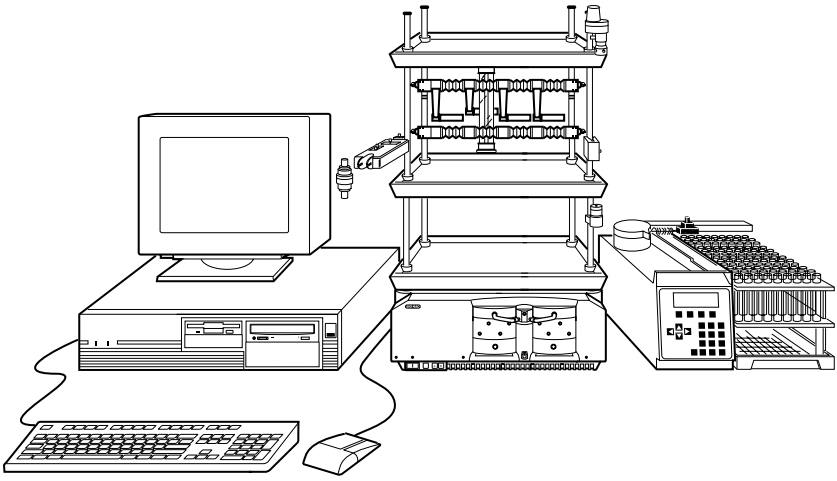

BioLogic Chromatography System

Starter Kit Instructions for the First Time User of the BioLogic Duo-Flow and BioLogic HR Systems

Catalog Number
750-0135

BIO-RAD

Table of Contents

Introduction

This instruction manual and starter kit contents may be used for both the BioLogic Duo-Flow and BioLogic HR Chromatography Systems.

To use the starter kit with the BioLogic Duo-Flow System, refer to Section 1. To use the starter kit with the BioLogic HR System, refer to Section 3.

1. Starter Kit Components

This starter kit contains the following items for running a separation.

- 50 ml of buffer A, 250 mM Tris buffer pH 8.1 (10x concentrate)
- 50 ml of buffer B, 250 mM Tris buffer pH 8.1 plus 5.0 M NaCl (10x concentrate)
- One vial of anion exchange protein standards (catalog number 125-0561)
- One 1 ml disposable sample injection syringe
- One 50 µl sample loop
- One UNO Q1 column (catalog number 720-0001)

The chromatographic separation for this kit requires approximately 6 minutes.

2. Other Materials You Will Need

- Filtered high quality water (*i.e.*, HPLC grade water)
- One 500 ml graduated cylinder
- One 1 L sidearm flask
- Stir bar and stir plate
- Vacuum source for degassing
- Two 500 ml bottles
- Fraction collection tubes, 13 x 100 mm (at least 14 tubes)
- 100 ml beaker

Section 1
Preparation for Use of the Duo-Flow System

When the BioLogic Duo-Flow System is turned on, the Manual Control Screen is displayed. (see Figure 1) This screen consists of a series of boxes containing instrument faceplates which provide direct control of the gradient pumps, a fraction collector, the UV and conductivity detector range settings and valves.

1. Priming the BioLogic Duo-Flow Gradient Pumps.

a. Immerse the gradient pump A and B inlet lines in a container of HPLC grade (filtered, degassed) or other high quality water.

b. Connect the syringe (supplied with the fittings kit) to the priming port of pump head A.

c. Turn the priming port counter-clockwise to open the seal. Gently withdraw the syringe plunger to draw water into the pump head.

d. Repeat this operation several times until no air bubbles are visible in the inlet tubing.

e. Tighten the priming port by turning it clockwise.

f. Repeat this priming procedure for pump head B.

2. Moving the Inject Valve to Purge Position.

To change the status of an automated inject valve AVR7-3, go to the Manual Screen displayed on the controller monitor.

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 L. For all other automated valves the default is position 1.

3. Purging the BioLogic Duo-Flow Pumps.

a. Make sure that the inject valve is in the Purge position.

b. Press the Purge buttons A and B on the front of the workstation. The gradient pumps will run and the indicator lights will flash green.

c. Run both pumps for 2 minutes and then press the purge buttons again to stop the pump.

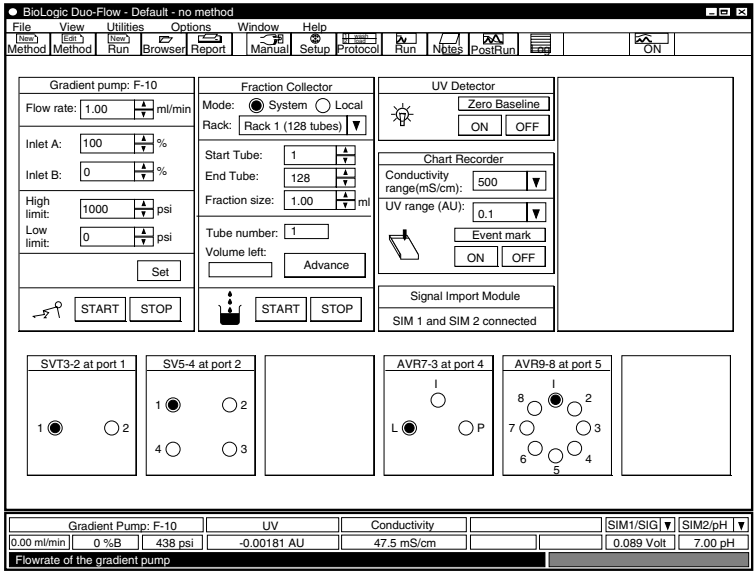

Fig. 1. Manual Control Screen.

4. Control of the BioLogic Duo-Flow Pumps.

The gradient pump parameters are set from the Manual Screen either by clicking in the appropriate field and entering a value from the keyboard or by using the spin arrows. You can set the flow rate between 0.1 to 10 ml/min and the gradient composition between 0% and 100% B.

To start the pump, click the **Start** button. Note the running man icon. To change the pump parameters while the pump is running, enter the new value and then click on the set button.

Pressure limits can be adjusted to match the pressure limits of a column. If the pressure limit is exceeded, the pump will stop and an alarm will sound. For now, set the high limit to 700 psi and the low limit to 20 psi.

5. Flushing the System through to the fraction collector.

With the gradient pumps stopped, move the inject valve back to position L (Load) by clicking radio button L (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 flow through the UV and conductivity flow cells and to the fraction collector, as described below.

Using a Model 2128 Fraction Collector

Connect the Model 2128 Fraction Collector by inserting the gray bus cable between it and the workstation. When it is connected, it will appear in the Manual Screen, as shown in Figure 2.

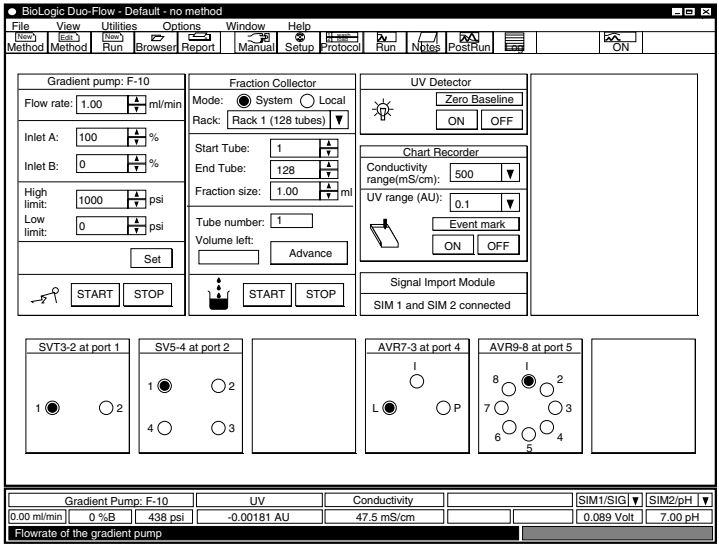

Fig. 2. Manual Control Screen showing Model 2128 Fraction Collector.

The Model 2128 Fraction Collector has two operating modes.

- System: Controlled by the BioLogic Duo-Flow System.
- Local: Controlled from its own faceplate.

For now, insure that the system button is selected.

When in System mode, the fraction collector operates as follows.

- Control of the Model 2128 is identical whether or not the optional on-arm diverter valve is used. Without a valve, the drop-head returns to the waste trough during divert operations.
- The fraction collector box on the Manual Screen will show fields for **Rack type**, **Start tube**, **End tube**, **Fraction size–ml**, **Tube number**, **Volume left**, a toggle button for **Start** and **Stop** and a button for **Advance** (see Figure 2).

6. Turning on the UV lamp.

a. The UV lamp automatically turns on when you turn on power to the workstation. (The UV lamp is turned off and on by clicking the On and Off buttons in the Manual Screen (see Figure 2). You should now check that the lamp is on; the mercury lamp requires approximately 30 minutes to warm up.

b. Clicking the **Zero Baseline** button will zero the UV signal.

7. The Manual Control Screen Chromatogram Window.

A feature of the Manual Screen is the ability to display a chromatogram window showing UV and conductivity values over a 10 minute interval. This is useful during column equilibration.

- To access this feature, simply click the Chromatogram Window **On** button, at the far right of the toolbar (See Figure 2). The chromatogram window will be displayed at the bottom of the screen, over the valve position indicators. The time axis is reset automatically at the end of 10 minutes or reset manually by clicking the **Clear Traces** button.

  Use the scroll bars to change the scaling of the axes.

- To close the Chromatogram Window, again click the toolbar button. The valve status indicators will reappear.

8. The Status Bar.

At the bottom of the Manual Screen is a status bar which is continually updated with system parameters.

Section 2
Anion Exchange Separation of Protein Standards

The starter kit allows you to learn how to use the BioLogic Duo-Flow System by programming and running a separation of a premixed anion exchange standard containing equine myoglobin, conalbumin, chicken ovalbumin and soybean trypsin inhibitor, using a 1.3 ml UNO Q1 Column*. Equine myoglobin is not retained on the UNO Q1 Column and elutes in the void volume. Conalbumin, chicken ovalbumin and soybean trypsin inhibitor bind to the column and require increased salt concentrations for elution. Separation requires approximately 6 minutes.

2.1 Overview of the Procedure

Run Conditions

- Buffer A = 25 mM Tris-HCl, pH 8.1
- Buffer B = 25 mM Tris-HCl, pH 8.1, 0.5M NaCl
- Flow rate 4.00 ml/min
- Sample volume 50 µl
- UV detection 0.1 AUFS
- Conductivity 100 mS/cm

General Procedure

Step 1 Buffer preparation

Step 2 Sample preparation

Step 3 Installation of the UNO Q1 column

Step 4 Priming the gradient pumps and equilibrating the column

Step 5 Writing a method

a) Programming the Setup Editor

b) Programming the Protocol Editor

c) The Run Control Screen

d) Starting a Run

* UNO Q1 Column: catalog number 720-0001

2.2 Buffer Preparation

Buffer A

1. Empty the contents of the bottle labeled buffer A into a 500 ml graduated cylinder and add filtered, high-quality water to 500 ml volume.
2. Place the contents of the graduated cylinder into a 1 L side-arm flask and drop in a stir bar. Cap the side arm flask, place it on a stir plate and connect it to a vacuum source. Degas the buffer for approximately 15 minutes with gentle stirring.
3 When degassing is complete, pour the buffer into a bottle and label appropriately.

Buffer B

Prepare buffer B by following the same procedure for preparation of buffer A.

2.3 Sample Preparation

1. Remove the aluminum cap from the anion exchange standards vial. Slowly remove the rubber plug from the standards vial (the contents may be under vacuum).
2. Add 1.0 ml of prepared buffer A to the vial.
3. Replace the rubber stopper and gently invert the vial to solubilize the protein standards.

2.4 Installation of the UNO Q1 column

Remove end caps from the UNO Q1 column. Keeping tubing lengths to a minimum, connect 1/16" tubing from port 4 of the inject valve to the column inlet. Connect the column outlet to the bottom of the UV flow cell. Secure the column in a vertical position.

2.5 Priming the Gradient Pumps and Equilibrating the UNO Q1 Column

Be sure the gradient pumps are stopped and the inject valve is in the purge position. Re-prime and purge pumps A and B as described in Section 2, step 1 of this manual.

Set the inject valve to position L (Load). Set the flow rate to 2.0 ml/min. Set the UV range to 0.1 AUFS and the conductivity range to 100 mS/cm.

1. Wash the column with 6.5 ml (5 column volumes) of buffer B at 2 ml/min to remove the column storage solution.
2. Equilibrate the column with13 ml (10 column volumes) of 100% buffer A.

   The conductivity monitor on the status bar should now read approximately 3 mS/cm.

2.6 Creating a Method

From the Manual Control screen, click the **New Method** toolbar button. You will see a dialog box asking for a method name (input Starter Kit Separation–Auto). Use the keyboard tab key to go to the Method Description and Method Author fields. Make an entry in these fields if desired and click the **OK** button. You will now see the Setup Editor.

Programming the Setup Editor

This information entered in the Setup Editor is important for the subsequent separation method. The buttons grouped in the left hand box (Available Devices) shows all the devices that could theoretically be connected to the BioLogic Duo-Flow System.

The list of items in the right hand box (Devices in Setup) identifies those devices you wish to use in the subsequent method. The initial default Devices in Setup are a UV detector and a conductivity monitor as these come as standard with the BioLogic Duo-Flow System. The default devices in Setup can be changed by choosing **Save setup as default** from the Data pull-down menu.

1. Click on the Fraction Collector button in the Available Devices box. A dialog box will appear asking you to choose the type of collector *i.e.* a generic collector, a Model 2110 or a Model 2128. Click on **Model 2128** and click the **OK** button.

   You will see 2128 Fraction Collector in the Devices in Setup box.

2. Next, click on the AVR7-3 Valve button in the Available Devices box. You will see a dialog box with a pull-down menu under **Valve Name/Function**. You should see **Sample Inject** already selected. Other functions are available from the pull-down menu. This action is called valve function aliasing. You will see that the valve position names are Load for position 1, Inject for position 2, and Purge for position 3.

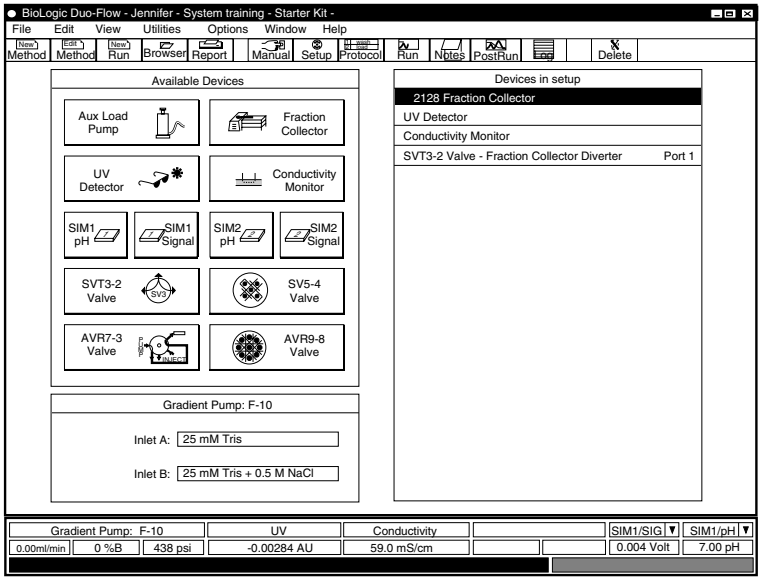

Fig. 3. Setup editor.

3. Next, go to the box labeled **Gradient Pump**. Use the tab key or click and drag on the field buffer A to highlight it and type in 25 mM Tris, pH 8.1. Use the tab key to highlight buffer B and type 25 mM Tris + 0.5 M NaCl, pH 8.1. This operation is called aliasing the gradient pump inlets.

4. The Setup Editor is now complete and you are now ready to program the separation steps. To see the protocol editor, click on the Protocol Editor toolbar menu button.

Programming the Protocol Editor

1. From the Options pull-down menu, insure that Use Volume (ml) is selected, so that the programming base is Volume.

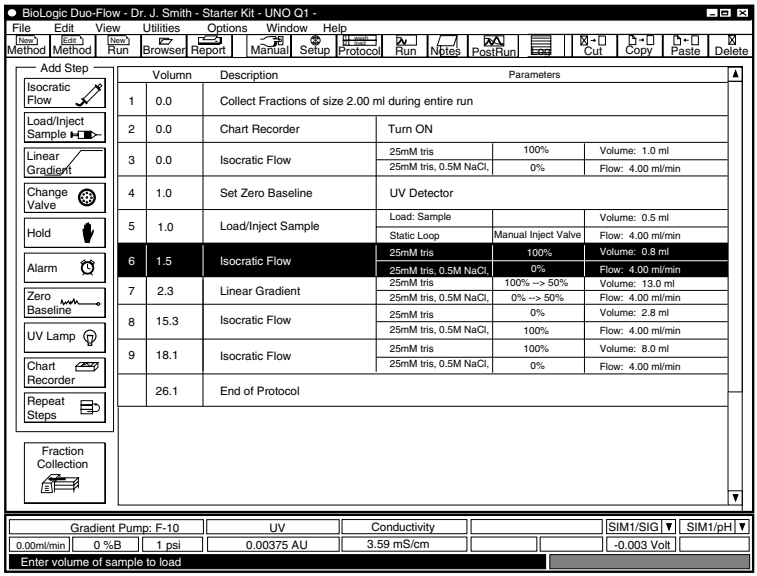

Fig. 4. Protocol editor.

2. Program the separation method listed below and in Figure 4.

   - From the left side of the screen, press the **Fraction collection** button. In the pop-up window that appears, choose **Collect All** with a fraction size of 2.00 ml and a delay of 0. Make sure that you choose the correct rack number for the rack you will be using with the Model 2128.

   - Program the remaining steps using the add step icons from the left-hand side of the screen.

Step Number	Start (ml)	Step
1.	0.0	Collect fractions of size 2.00 ml during entire run
2.	0.0	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 1.0 ml
3.	1.0	Set UV baseline to 0.0
4.	1.0	Static loop: Inject 0.5 ml sample at 4.00 ml/min for 0.1 min
5.	1.5	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 0.8 ml
6.	2.3	Linear gradient with 0% to 50% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 13.0 ml
7.	15.3	Isocratic flow with 0% 25 mM tris, pH 8.1, 100% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 2.8 ml
8.	18.1	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 8.0 ml
9.	26.1	End of protocol

3. When you have finished programming the Protocol Editor, click on the toolbar button RUN. You will see a dialog box asking you to name the run. For now, type in Run 1 and click the OK button. You will now see the Run Control Screen (see Figure 5).

The Run Control Screen

1. Use the toolbar buttons at the left-hand side of the screen to check that the screen display ranges for UV (0.1 AUFS) and conductivity (100 mS/cm) are correctly set and that the gradient pump pressure limits are appropriate (set to 700 psi High and 20 psi Low).
2. If you were equilibrating the column while writing the method, you will notice that the Status Bar is displaying the flow-rate and values for UV and conductivity. If necessary, you may wish to zero the UV trace by clicking on the **Zero baseline** button. This button may be clicked at any time.
3. To scale the on-screen chromatogram display axes, use the scroll bars located on the left and right axes of the chromatogram window.

Starting the Run

1. Insure that sufficient tubes are in the fraction collector rack (approximately 14). The drophead will automatically move to tube 1 when the Run is started.
2. Insure that the AVR7-3 valve is in the LOAD position (position L). If it is not, return to the Manual Screen by clicking the toolbar **Manual** button and click on valve position L.

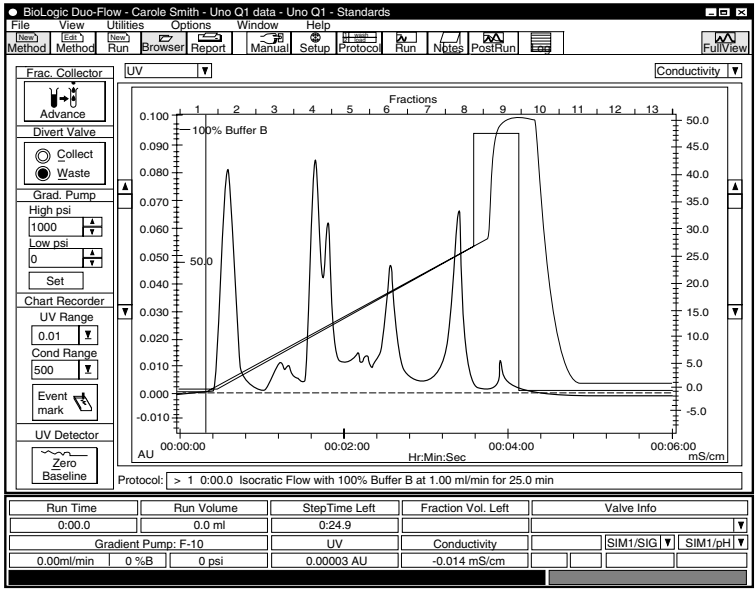

Fig. 5. Run control screen.

3. Insure that the 50 µl sample loop is connected to ports 3 and 6 of the inject valve. Completely fill the loop with sample protein standard via port 5 and a syringe and needle. **Do not** remove the syringe from the injection port after filling the loop or the sample will siphon to waste.

4. To launch the Run, click on the green **Start** toolbar button. The sample will be loaded automatically.

5. Clicking the **Hold** toolbar button will hold the gradient pumps at the current %B value and will not advance the programmed method until the **Continue** toolbar button is pressed. Clicking the **Pause** toolbar button will stop the pumps completely. Clicking the **Continue** toolbar button will re-start the pumps at exactly the point where the program was paused.

6. When this run is finished, the pumps automatically stop and a run finished message appears in the bottom right of the status bar.

7. Figure 5 shows a typical run screen and chromatogram for this separation.

Section 3
Preparation for Use of the BioLogic HR System

The procedures in this document are written for two configurations, which are distinguished by the following.

- Manual: Uses a V7-3 Manual Inject Valve, a Model 2110 Fraction Collector, and an SV3-2 Diverter Valve.
- Automated: Uses an AV7-3 Automatic Inject Valve and a Model 2128 Fraction Collector.

When you turn on the BioLogic HR System, the Manual Control Screen is displayed (see Figure 6). This screen consists of a series of boxes containing instrument faceplates which provide direct control of the gradient pumps, a fraction collector, a chart recorder (apart from chart speed, which is set on the recorder itself), the UV and conductivity detector range settings and solenoid and automated valves.

1. Priming the BioLogic HR Gradient Pumps.

a. Immerse the gradient pump A and B inlet lines in a container of HPLC grade (filtered, degassed) or other high quality water.

b. Connect the syringe (supplied with the fittings kit) to the priming port of pump head A.

c. Turn the priming port counter-clockwise to open the seal. Gently withdraw the syringe plunger to draw water into the pump head.

d. Repeat this operation several times until no air bubbles are visible in the inlet tubing.

e. Tighten the priming port by turning it clockwise.

f. Repeat this priming procedure for pump head B.

2. Moving the Inject Valve to Purge Position.

- For a manual inject valve V7-3, move the arm to the Purge position (left, then up, then left). The figure in the valve position window displays **P**.

  Flow from the gradient pumps will now go directly to waste, bypassing any column or detector downstream of the valve.

- To change the status of an automated inject valve AV7-3, go to the Manual Screen displayed on the controller monitor.

If you plugged an inject valve into port 4, you will see a valve box designated **AV7-3 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 AV7-3 valve to Purge position, click button **P**.

Note: The default position at power up and at the end of a programmed method for the AV7-3 is L and for all other automated valves is position 1.

3. Purging the BioLogic HR Pumps.

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

a. Press the Purge buttons A and B on the front of the Workstation. The gradient pumps will run and the indicator lights will flash green. The default purge flow rate is 7 ml/min per pumphead, although this may be changed using the options (manual setup) pull-down menu.

b. Run both pumps for 2 minutes and then press the Purge buttons again to stop the pump.

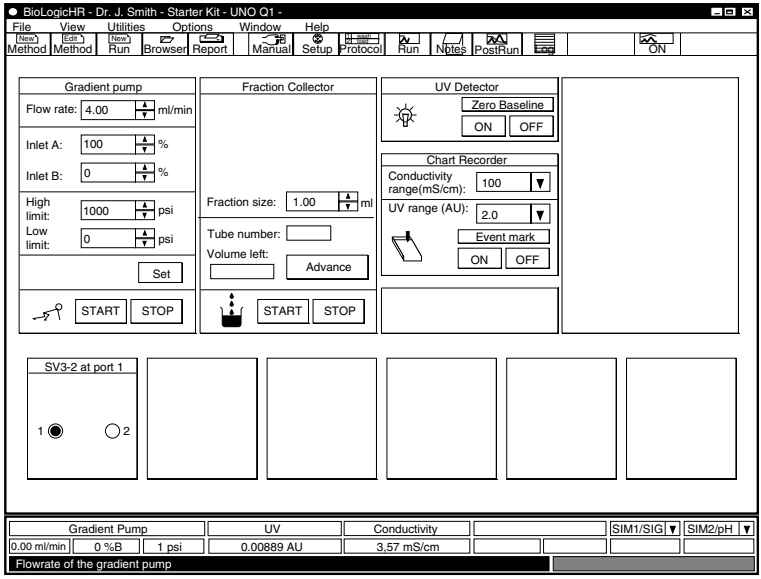

Fig. 6. Manual control screen for a Manual BioLogic HR System.

4. Control of the BioLogic HR Pumps.

The gradient pump parameters are set from the manual screen either by clicking in the appropriate field and entering a value from the keyboard or by using the spin arrows. You can alter the flow rate from 0.1 to 10 ml/min for any given proportion of pumps A and B. Note that the total flow rate can not exceed 10 ml/min (*i.e.* with a set flow-rate of 10 ml/min at 50% B, both pump heads A and B are delivering at 5 ml/min each).

To start the pump, click the **Start** button. Note the running man icon. To change the pump parameters while the pump is running, enter the new value and then click on the **Set** button.

The maximum pressure capability of the BioLogic HR gradient pumps is 1000 psi. Pressure limits can be adjusted to match the pressure limits of a column. If the pressure limit is exceeded, the pump will stop and an alarm will sound. For now, set the high limit to 700 psi and the low limit to 20 psi.

5. Flushing the System through to the fraction collector.

With the gradient pumps stopped, move the inject valve back to position **L** (Load) by either moving the lever to the right (V7-3) or clicking radio button **L** (AV7-3) on the Manual Screen.

Using the Manual Screen, set the pump flow rate at 1.0 ml/min and start the pump. Water will flow through the UV and conductivity flow cells and to the fraction collector, as described below.

a. Using the Model 2110 Fraction Collector and SV3-2 valve as a diverter valve.

If you plugged the SV3-2 valve into Solenoid valve port 1, you will see a valve box on the Manual Screen designated **SV3-2 at port 1**. (see Figure 2) The two radio buttons of this box correspond to valve positions; 1 = Waste, 2 = Collect. Select position 1 (waste) to divert the flow to waste. The fraction collector box on the manual screen (refer to Figure 2) will show fields for **fraction size–ml**, **Tube number**, **Volume left**, a toggle button for **Start** and **Stop** and a button for **Advance**. Note that tube number is a display field only.

- Place tubes in the fraction collector rack and set a fraction size of 1 ml. Click on the **Start** button and select valve position 2 of the SV3-2 valve box. Flow will now be diverted to the fraction collector tubes. Click the **Advance** button to test its function if desired.

- Continue to pump water through the system for at least 10 minutes. You may wish to continue fraction collection or return the SV3-2 valve to position 1 and then stop fraction collection.

Note: In **manual** operation, starting or stopping fraction collection does not change the SV3-2 diverter valve position. You must always manually set the SV3-2 valve to the waste or collect position.

b. Using a Model 2128 Fraction Collector.

The Model 2128 Fraction Collector has two operating modes:

- System: Controlled by the BioLogic HR System.
- Local: Controlled from its own faceplate.

For now, insure that the System button is selected.

When in System mode, operation of the fraction collector is as follows.

- Control of the Model 2128 is identical regardless of whether or not the optional on-arm diverter valve is used. Without a valve, the drop-head returns to the waste trough during divert operations.
- The fraction collector box on the manual screen will show fields for **Rack type**, **Start tube**, **End tube**, **Fraction size–ml**, **Tube number**, **Volume left**, a toggle button for **Start** and **Stop** and a button for **Advance** (see Figure 7).

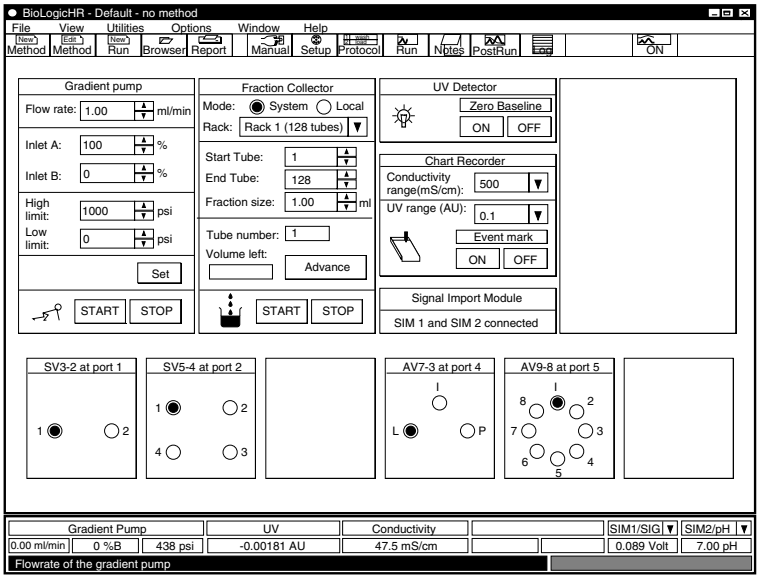

Fig. 7. Manual Control Screen for the Automated BioLogic System.

6. **Turning on the UV lamp.**

   a. The UV lamp automatically turns on when you turn on power to the workstation. The UV lamp is turned off and on by clicking on the toggle button. You should now check that the lamp is on; the mercury lamp requires approximately 30 minutes to warm up.

   b. Clicking the **Zero Baseline** button will zero the UV signal to the recorder and to the on-screen display.

7. **Setting the UV and Conductivity Detector ranges on the Chart Recorder.**

   a. The UV Detector range is set by clicking on a value (from 2.0 to 0.001 AUFS) from the drop-down menu (refer to Figure 2). Note that this range setting applies only to the output to a chart recorder. On-screen displays of the UV range are controlled by the scroll bars and have no effect on the output to a chart recorder.

      For now, set the scale to 0.1 AUFS.

   b. The conductivity monitor range is set by clicking on a value (from 0.10 to 500.0 mS/cm) from the drop-down menu. Note that this range of settings applies only to the output to a chart recorder. On-screen displays of the conductivity range are controlled by the scroll bars and have no effect on the output to a chart recorder.

      For now, set the scale to 100 mS/cm.

8. **The Manual Control Screen Chromatogram Window.**

   A feature of the Manual Screen is the ability to display a chromatogram window showing UV and conductivity values over a 10 minute time axis. This is useful during column equilibration.

   - To access this feature, simply click on the chromatogram window toolbar button at the far right of the toolbar. The chromatogram window will be placed over the valve panels. The time axis is reset automatically at the end of 10 minutes or reset manually by clicking the **Clear Traces** button.

     Use the scroll bars to change the scaling of the axes.

   - To close the chromatogram window, again click the toolbar button. The valve panels will reappear.

9. Controlling the chart recorder.

Insure that the voltage inputs on the recorder are set at 1V.

- The chart recorder pen up/down and chart stop/start functions are controlled by the **ON/OFF** toggle switch, which is displayed in the Manual screen.
- Chart speed is set at the chart recorder.
- Clicking the **Event Mark** button will send an event mark to the recorder.

10. The Status Bar.

At the bottom of the Manual Screen is a status bar which is continually updated with system parameters.

Section 4
Anion Exchange Separation of Protein Standards

The starter kit allows you to learn how to use the BioLogic HR System by programming and running a separation of a premixed anion exchange standard containing equine myoglobin, conalbumin, chicken ovalbumin and soybean trypsin inhibitor, using a 1.3 ml UNO Q1 Column*. Equine myoglobin is not retained on the UNO Q1 Column and elutes in the void volume. Conalbumin, chicken ovalbumin and soybean trypsin inhibitor bind to the column and require increased salt concentrations for elution. Separation requires approximately 6 minutes.

4.1 Overview of the Procedure

Run Conditions

- Buffer A = 25 mM Tris-HCl, pH 8.1
- Buffer B = 25 mM Tris-HCl, pH 8.1, 0.5M NaCl
- Flow rate 4.00 ml/min
- Sample volume 50 µl
- UV detection 0.1 AUFS
- Conductivity 100 mS/cm
- Chart speed 60 cm/hr

General Procedure

Step 1 Buffer Preparation

Step 2 Sample Preparation

Step 3 Installation of the UNO Q1 Column

Step 4 Priming the gradient pumps and equilibrating the column

Step 5 Writing a method for the Manual BioLogic HR System

a) Programming the Setup Editor

b) Programming the Protocol Editor

c) The Run Control Screen

d) Starting a Run

Step 6 Writing a method for the Automated BioLogic HR System

a) Programming the Setup Editor

b) Programming the Protocol Editor

c) The Run Control Screen

d) Starting a Run

* UNO Q1 Column: catalog number 720-0001

4.2 Buffer Preparation

Buffer A

1. Empty the contents of the bottle labeled buffer A into a 500 ml graduated cylinder and add filtered, high-quality water to 500 ml volume.
2. Place the contents of the graduated cylinder into a 1 L side-arm flask and drop in a stir bar. Cap the side arm flask, place it on a stir plate and connect it to a vacuum source. Degas the buffer for approximately 15 minutes with gentle stirring.
3 When degassing is complete, pour the buffer into a bottle and label appropriately.

Buffer B

Prepare buffer B by following the same procedure for preparation of buffer A.

4.3 Sample Preparation

1. Remove the aluminum cap from the anion exchange standards vial. Slowly remove the rubber plug from the standards vial (the contents may be under vacuum).
2. Add 1.0 ml of prepared buffer A to the vial.
3. Replace the rubber stopper and gently invert the vial to solubilize the protein standards.

4.4 Installation of the UNO Q1 Column

Remove end caps from the UNO Q1 column. Keeping tubing lengths to a minimum, connect 1/16" tubing from port 7 of the inject valve to the column inlet. Connect the column outlet to the bottom of the UV flow cell. Secure the column in a vertical position.

4.5 Priming the Gradient Pumps and Equilibrating the UNO Q1 Column

Be sure the gradient pumps are stopped and the inject valve is in the purge position. Re-prime and purge pumps A and B as described in Section 2, step 1 of this manual.

Set the inject valve to position L (Load). Set the flow rate to 2.0 ml/min. Set the UV range to 0.1 AUFS and the conductivity range to 100 mS/cm.

1. Wash the column with 6.5 ml (5 column volumes) of buffer B at 2 ml/min to remove the column storage solution.
2. Equilibrate the column with13 ml (10 column volumes) of 100% buffer A.

   The conductivity monitor on the status bar should now read approximately 3 mS/cm.

4.6 Creating a Method for the Manual BioLogic HR System

From the Manual Control Screen, click on the **New Method** toolbar button. In the pop-up window which appears, input the method name Starter Kit Separation–Manual. Use the keyboard's tab key to step through the Method Description and Method Author fields. Make an entry in these fields if desired and click the **OK** button. You will now see the Setup Editor Screen.

On the chart recorder, set the chart speed at 60 cm/hr.

Note: Chart speed is not controlled at the BioLogic HR System.

Programming the Setup Editor

The information entered in the setup editor screen is important for the subsequent separation method. The buttons grouped in the left hand box (Available Devices) show all the devices that could theoretically be connected to the BioLogic HR System.

The list of items in the right hand box (Devices in Setup) identifies those devices you wish to use in the subsequent method. The initial default Devices in Setup are a UV detector and a conductivity monitor as these come as standard with the BioLogic HR System. The default devices in Setup can be changed by choosing **Save setup as default** from the Data pull-down menu.

Note: The following procedure is for a Manual BioLogic HR System. Refer to Section 4.7 for the Automated BioLogic HR System setup procedure.

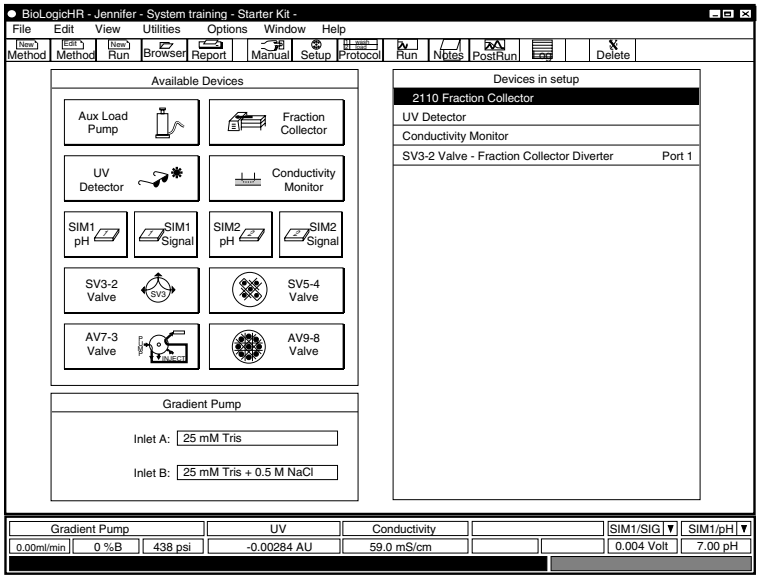

Fig. 8. Setup Editor for a Manual BioLogic HR System.

1. Click on the fraction collector button in the Available Devices box. A dialog box will appear asking you to choose the type of collector (a generic collector, a Model 2110 or a Model 2128). Click on **Model 2110** and click the **OK** button.

   You will now see **2110 Fraction Collector** in the Devices in Setup box (see Figure 3).

2. Next, click on the SV3-2 valve button in the Available Devices box. You will see a dialog box with a pull-down menu under **Valve Name/Function**. You should see **Fraction Collector Diverter** already selected. Other functions are available from the pull-down menu. This action is called aliasing. You will see that the valve position names are Waste for position 1 and Collect for position 2.

   Note that the functioning of this particular valve is now automatically tied to the programmed operation of the fraction collector during a Method (but does not affect the manual screen operation).

3. Next, go to the box labeled **Gradient Pump**. Use the tab key or click and drag on the field buffer A to highlight it and type in 25 mM Tris, pH 8.1. Use the tab key to highlight buffer B and type 25 mM Tris + 0.5 M NaCl, pH 8.1. This operation is called aliasing the gradient pump inlets.

4. The Setup Editor is now complete and you are now ready to program the separation steps. To see the Protocol Editor, click on the Protocol Editor toolbar menu button.

Programming the Protocol Editor

Figure 9 shows the Protocol Editor programming screen.

1. From the Options pull-down menu, insure that Use Volume (ml) is selected, so that the method is based on Volume.

2. Program the separation method presented in the table on the following page.

   - From the left side of the screen, click on the **Fraction Collection** button. In the pop-up window that appears, choose **Collect All** with a fraction size of 2.00 ml and a delay of 0.

   - Program the remaining steps by selecting the appropriate Add Step icons from the left side of the screen.

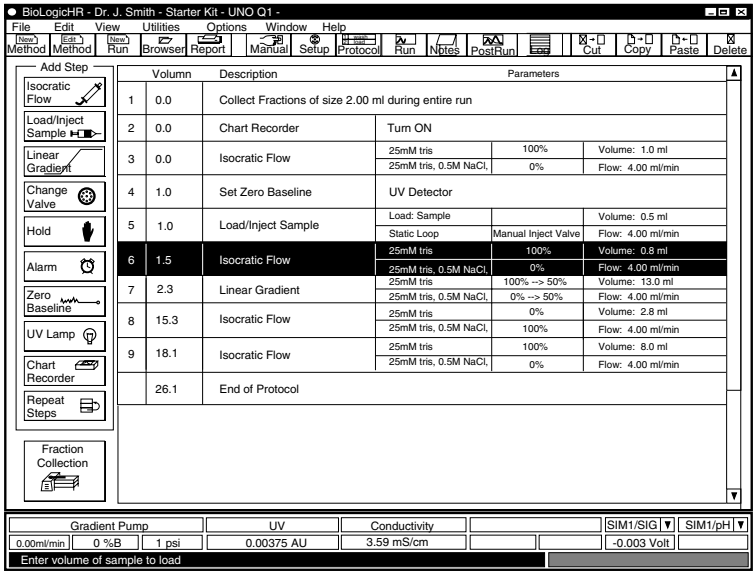

Fig. 9. Protocol Editor for a Manual BioLogic HR System.

Step Number	(ml) Start	Step
1.	0.0	Collect fractions of size 2.00 ml during entire run
2.	0.0	Turn chart recorder ON
3.	0.0	Isocratic Flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 1.0 ml
4.	1.0	Set UV baseline to 0.0
5.	1.0	Static loop: Inject 0.5 ml sample at 4.00 ml/min for 0.1 min
6.	1.5	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 0.8 ml
7.	2.3	Linear gradient with 0% to 50% 25 mM tris 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 13.0 ml
8.	15.3	Isocratic flow with 0% 25 mM tris, pH 8.1, 100% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 2.8 ml
9.	18.1	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 8.0 ml
10.	26.1	End of protocol

3. When you have finished with the protocol editor, click on the toolbar button **RUN**. You will see a dialog box asking you to name the run. For now, type in Run 1 and click the **OK** button. You will now see the Run Control Screen (see Figure 10).

The Run Control Screen

1. Use the toolbar buttons at the left side of the screen to check that the chart recorder ranges for UV (0.1 AUFS) and conductivity (100 mS/cm) are correctly set and that the gradient pump pressure limits are appropriate (set to 700 psi High and 20 psi Low).

2. If you were equilibrating the column while writing the method, you will notice that the status bar is displaying the flow-rate and values for UV and conductivity. If necessary, you may wish to zero the UV trace by clicking on the **Zero baseline** button. This button may be clicked at any time.

3. To scale the on-screen chromatogram display axes, use the scroll bars located on the left and right axes of the chromatogram window.

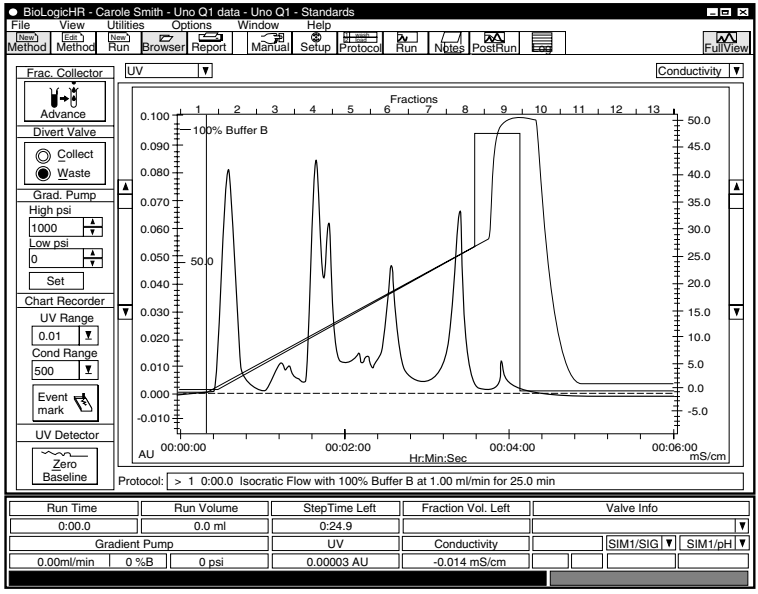

Fig. 10. Run Control Screen showing a completed run on a Manual BioLogic HR System.

Starting the Run

1. Insure that sufficient tubes are in the fraction collector rack (approximately 14) and that the drop-head is over tube 1.
2. Insure that the V7-3 valve is in the LOAD position (position L).
3. Insure that the 50 µl sample loop is connected to ports 3 and 6 of the inject valve. Completely fill the loop with sample (anion exchange protein standard) via port 5 and a syringe and needle. **Do not** remove the syringe from the injection port after filling the loop or the sample will siphon to waste.
4. Click on the green **Start** toolbar button to start the method. The alarm will sound after 1 minute. At this time move the V7-3 valve to the Inject position (*i.e.* move the arm to the left). The sample will be loaded onto the column and the method will be advanced. You may choose to leave the valve in the inject position for the duration of the run or return it to load position after all the sample has been loaded.
5. To Hold, Pause or Abort a run, click on the appropriate toolbar button. Clicking the **Hold** toolbar button will hold the gradient pumps at the current %B value and will not advance the programmed Method until the **Continue** toolbar button is pressed. Clicking the **Pause** toolbar button will stop the pumps completely. Clicking the **Continue** toolbar button will re-start the pumps at exactly the point where the program was paused.
6. When this run is finished, the gradient pumps will automatically stop and a run finished message will appear in the bottom right of the status bar.
7. Figure 10 shows a typical run control screen and chromatogram for this separation.

4.7 Creating a Method for the Automated BioLogic HR System

From the Manual Control Screen, click the **New Method** toolbar button. You will see a dialog box asking for a method name (input Starter Kit Separation–Auto). Use the keyboard tab key to go to the Method Description and Method Author fields. Make an entry in these fields if desired and click the **OK** button. You will now see the Setup Editor.

At the chart recorder, set the Chart Speed at 60 cm/hr.

Note: Chart speed is not controlled at the BioLogic HR system.

Programming the Setup Editor

This information entered in the Setup Editor (see Figure 11) is important for the subsequent separation method. The buttons grouped in the left hand box (Available Devices) shows all the devices that could theoretically be connected to the BioLogic HR System.

The list of items in the right hand box (Devices in Setup) identifies those devices you wish to use in the subsequent method. The initial default Devices in Setup are a UV detector and a conductivity monitor as these come as standard with the BioLogic HR System. The default devices in Setup can be changed by choosing **Save setup as default** from the Data pull-down menu.

1. Click on the fraction collector button in the available devices box. A dialog box will appear asking you to choose the type of collector *i.e.* a generic collector, a Model 2110 or a Model 2128. Click on **Model 2128** and click the **OK** button.

   You will now see 2128 Fraction Collector in the devices in setup box.

2. Next, click on the AV7-3 Valve button in the Available Devices box. You will see a dialog box with a pull-down menu under **Valve Name/Function**. You should see **Sample Inject** already selected. Other functions are available from the pull-down menu. This action is called valve function aliasing. You will see that the valve position names are Load for position 1, Inject for position 2, and Purge for position 3.

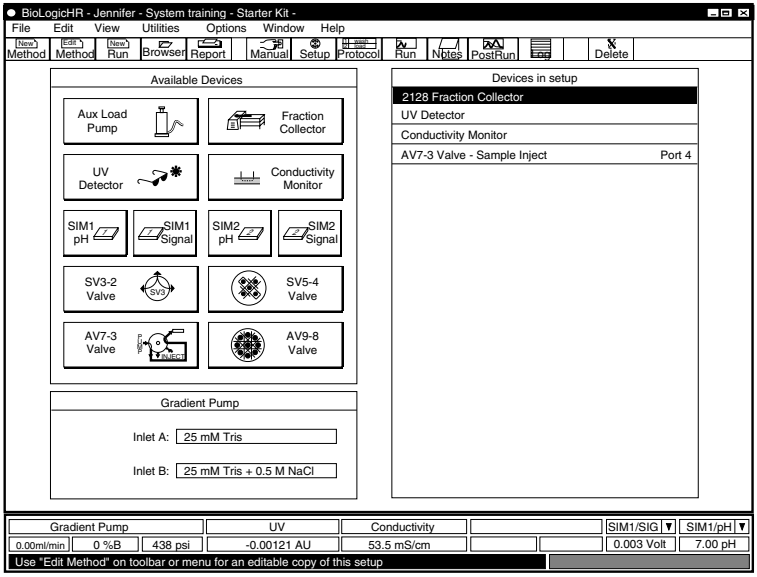

Fig. 11. Setup editor for an Automated BioLogic HR System.

3. Next, go to the box labeled **Gradient Pump**. Use the tab key or click and drag on the field buffer A to highlight it and type in 25 mM Tris, pH 8.1. Use the tab key to highlight buffer B and type 25 mM Tris + 0.5 M NaCl, pH 8.1. This operation is called aliasing the gradient pump inlets.

4. The Setup Editor is now complete and you are now ready to program the separation steps. To see the Protocol Editor, click on the Protocol Editor toolbar menu button.

Programming the Protocol Editor

1. From the Options pull-down menu, insure that Use Volume (ml) is selected, so that the programming base is Volume.

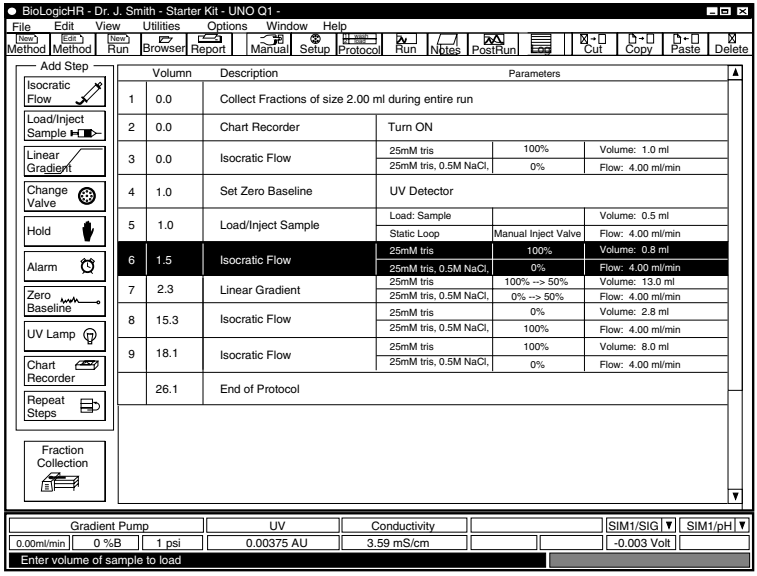

Fig. 12. Protocol editor for an Automated BioLogic System.

2. Program the separation method listed below and in Figure 12.

- From the left side of the screen, press the **Fraction Collection** button. In the pop-up window that appears, choose **Collect All** with a fraction size of 2.00 ml and a delay of 0. Make sure that you choose the correct rack number for the rack you will be using with the model 2128.
- Program the remaining steps using the add step icons from the left side of the screen.

Step Number	Start (ml)	Step
1.	0.0	Collect fractions of size 2.00 ml during entire run
2.	0.0	Turn chart recorder ON
3.	0.0	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 1.0 ml
4.	1.0	Set UV baseline to 0.0
5.	1.0	Static loop: Inject 0.5 ml sample at 4.00 ml/min for 0.1 min
6.	1.5	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 0.8 ml
7.	2.3	Linear gradient with 0% to 50% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 13.0 ml
8.	15.3	Isocratic flow with 0% 25 mM tris, pH 8.1, 100% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 2.8 ml
9.	18.1	Isocratic flow with 100% 25 mM tris, pH 8.1, 0% 25 mM tris, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 8.0 ml
10.	26.1	End of protocol

3. When you have finished programming the Protocol Editor, click on the toolbar button **RUN**. You will see a dialog box asking you to name the run. For now, type in Run 1 and click the **OK** button. You will now see the Run Control Screen (see Figure 13).

The Run Control Screen

1. Use the toolbar buttons at the left side of the screen to check that the chart recorder ranges for UV (0.1 AUFS) and conductivity (100 mS/cm) are correctly set and that the gradient pump pressure limits are appropriate (set to 700 psi High and 20 psi Low).
2. If you were equilibrating the column while writing the Method, you will notice that the status bar is displaying the flow-rate and values for UV and conductivity. If necessary, you may wish to zero the UV trace by clicking on the **Zero baseline** button. This button may be clicked at any time.
3. To scale the on-screen chromatogram display axes, use the scroll bars located on the left and right axes of the chromatogram window.

Starting the Run

1. Insure that sufficient tubes are in the fraction collector rack (approximately 14). The drophead will automatically move to tube 1 when the run is started.
2. Insure that the AV7-3 valve is in the LOAD position (position L). If it is not, return to the Manual Screen by clicking the toolbar **Manual** button and click on valve position L.

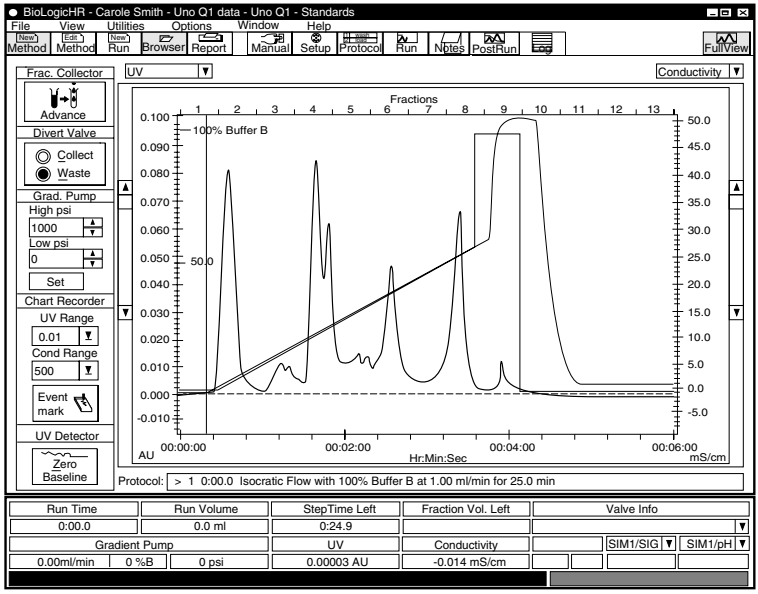

Fig. 13. Run control screen showing a completed run on an Automated BioLogic HR System.

3. Insure that the 50 µl sample loop is connected to ports 3 and 6 of the inject valve. Completely fill the loop with sample protein standard via port 5 and a syringe and needle. **Do not** remove the syringe from the injection port after filling the loop or the sample will siphon to waste.

4. To launch the Run, click on the green **Start** toolbar button. The sample will be loaded automatically.

5. Clicking the **Hold** toolbar button will hold the gradient pumps at the current %B value and will not advance the programmed method until the **Continue** toolbar button is pressed. Clicking the **Pause** toolbar button will stop the pumps completely. Clicking the **Continue** toolbar button will re-start the pumps at exactly the point where the program was paused.

6. When this run is finished, the pumps automatically stop and a run finished message appears in the bottom right of the status bar.

7. Figure 13 shows a typical run screen and chromatogram for this separation.

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