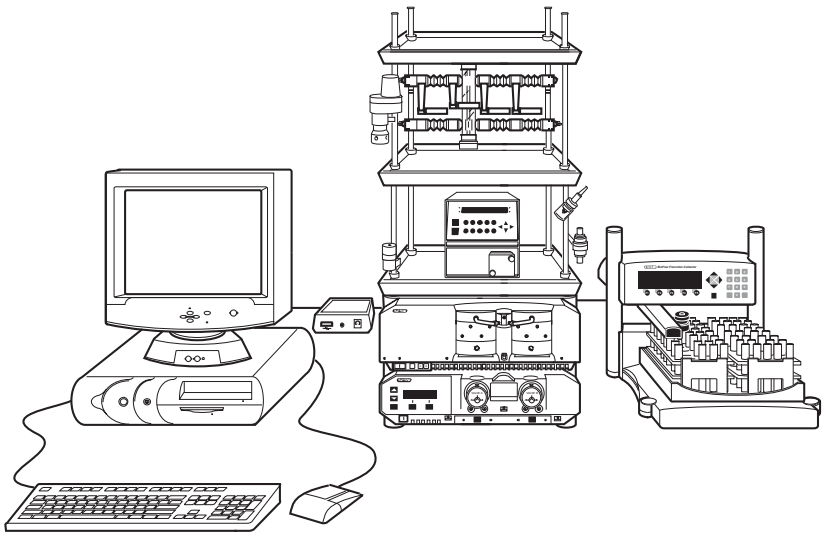
BioLogic DuoFlow™ Chromatography System Starter Kit

Instruction Manual

Catalog # 760-0135



BIO-RAD

Table of Contents

Introduction

This instruction manual and starter kit contents may be used for the BioLogic DuoFlow system and the BioLogic DuoFlow Maximizer™ and Pathfinder™ chromatography systems. The use of the starter kit with these systems is described in Sections 1 and 3, respectively.

1. Starter Kit Components

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

- 50 ml of buffer A, 250 mM Tris-HCl buffer, pH 8.1 (10x concentrate)
- 50 ml of buffer B, 250 mM Tris-HCl buffer, pH 8.1, plus 5.0 M NaCl (10x concentrate)
- 50 ml of Maximizer solution A1, 500 mM Tris-HCl (10x concentrate)
- 50 ml of Maximizer solution A2, 500 mM Tris base (10x concentrate)
- 50 ml of Maximizer solution B2, 5.0 M NaCl (2.5 x concentrate)
- One vial of anion exchange protein standard (catalog #125-0561)
- One 1 ml disposable sample injection syringe
- One 50 µl sample loop

The chromatographic separation for this kit requires approximately 6 minutes.

2. Materials You Will Need

In order to prepare the starter kit buffer solutions you will need the following materials:

- Filtered high-quality water (i.e., HPLC grade water)
- One 500 ml graduated cylinder
- One 1 L side-arm flask
- Stirbar and stirplate
- Vacuum source for degassing
- Two 500 ml bottles
- Fraction collection tubes, 13 x 100 mm (at least 14 tubes)
- 100 ml beaker

If you are using the DuoFlow Maximizer or Pathfinder systems you will also need the following materials:

- pH 7.00 and pH 10.00 standard buffer
- One 200 ml graduated cylinder (optional)
- Two additional 500 ml bottles
- Fraction collection 1.5 or 2 ml micro tubes

I. BioLogic DuoFlow System

Section 1. DuoFlow System Preparation

When the DuoFlow system is turned on, the Manual screen is displayed (see Figures 1 and 2). This screen displays instrument control panels that provide direct control of the pumps, valves, fraction collector, UV detector, QuadTec™ UV/Vis detector, and Econo™ Gradient Pump. The arrow button in the upper right corner of the detector control panel toggles between the UV and QuadTec detector control panels. Only those instruments connected to the system will be displayed.

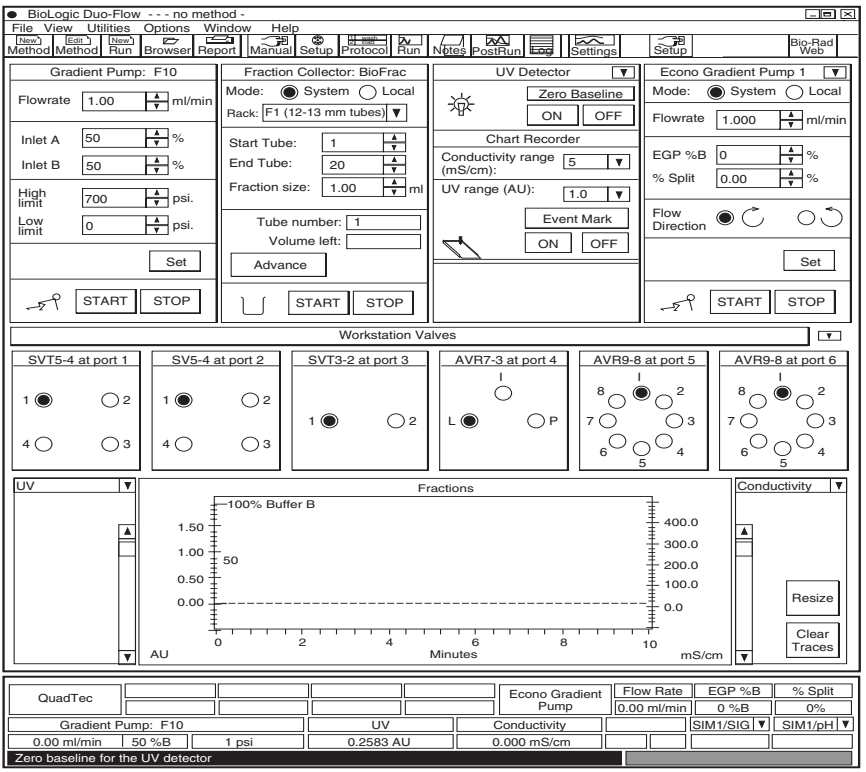


Fig. 1. Manual Control Screen with UV detector

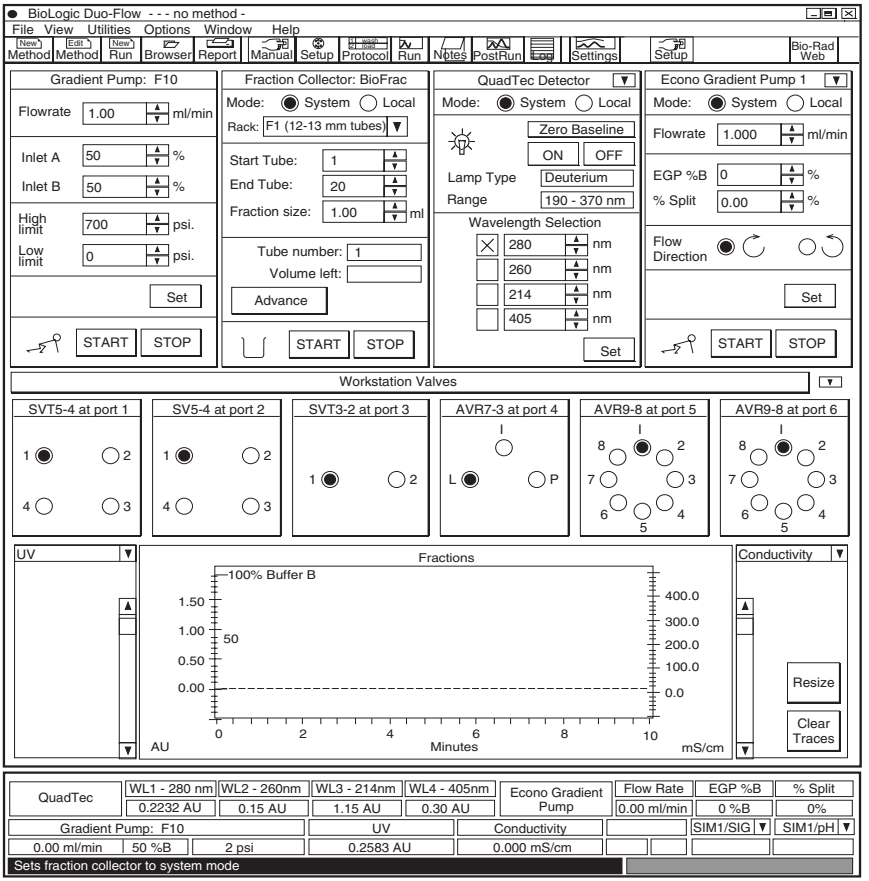


Fig. 2. Manual Control Screen with QuadTec detector

1.1 Prime the Workstation Pumps

a. Immerse the workstation 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 A.

c. Turn the priming port counter-clockwise one full turn 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 B.

1.2 Move the AVR7-3 Inject Valve to the Purge Position

Prior to purging the pumps at 10 ml/min it is essential to place the AVR7-3 valve in the purge position. This directs the flow to waste and not to the column and detector.

To change the position of the AVR7-3 inject valve, select P from the Manual screen valve control panel for the AVR7-3 valve. If you plugged the AVR7-3 inject valve into port 4 on the workstation rear panel, you will see a valve box designated AVR7-3 at port 4. The three 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.

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.

1.3 Purge the Workstation Pumps

a. Make sure that the AVR7-3 inject valve is in the Purge position.

b. Press the Purge buttons A then B on the front of the workstation. The workstation pumps will run at a default flow rate of 10 ml/min and the indicator light will flash green.

c. Run each pump for 2 minutes. Press the purge buttons again to stop the pump.

1.4 Manual Control of the Workstation Pumps

The workstation 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 arrows. You can set the flow rate between 0.01 to 10 ml/min and the gradient composition between 0 and 100% B.

To start the pump, click the Start button. Note that the running man icon will start running. 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. If you are using an UNO™ Q1 column, set the high limit to 700 psi and the low limit to 20 psi.

1.5 Flush the System Through to the Fraction Collector

With the gradient pumps stopped, move the AVR7-3 valve back to position L (Load) by clicking L (AVR7-3) on the Manual screen.

From the gradient pump control panel on the Manual screen, set the pump flow rate to 1.0 ml/min and start the pump. Water will flow through the UV or QuadTec and conductivity flow cells to the fraction collector, as described below.

BioFrac™ Fraction Collector

The BioFrac fraction collector has two operating modes:

- System—Controlled by the DuoFlow system
- Local—Controlled from its own faceplate in stand-alone mode

Ensure that the System button is selected.

When in System mode, the fraction collector control panel will show fields for Rack type, Start tube, End tube, Fraction size, Tube number, Volume left, a toggle button for Start and Stop, and a button for Advance (see Figures 1 and 2).

1.6 Turn on the UV lamp

DuoFlow UV detector

a. The UV lamp automatically turns on when you turn on power to the workstation. The UV lamp can be turned on and off by clicking the On and Off buttons from the UV detector control panel on the Manual screen (see Figure 1). Check that the lamp is on; the mercury lamp requires approximately 30 minutes to warm up.

b. Click the Zero Baseline button to zero the UV signal. The Status bar along the bottom of the screen provides AU output for the detector. Ensure that it goes to zero when you select the zero baseline option.

QuadTec UV/Vis detector

a. The QuadTec detector should be powered On before starting the BioLogic software. If the QuadTec detector is not powered up, exit the software, power up the QuadTec detector and restart the software. When connection is completed, “SLAVE” appears in the corner of QuadTec faceplate. The QuadTec appears in its own control panel as shown in Figure 2.

b. From the QuadTec detector control panel on the Manual screen (Figure 2), set the four wavelengths of the QuadTec detector to 280, 260, 214, and 405 nm. Select Set. The active wavelengths will appear in the lower screen status bar.

c. Click the Zero Baseline button to zero the four UV/Vis signals. The Status bar along the bottom of the screen provides AU output for the detector. Ensure that it goes to zero when you select the zero baseline option.

1.7 Manual Screen Chromatogram Window

A feature of the Manual screen is its ability to display up to eight traces of a chromatogram; including UV/Vis, pH, conductivity, %Buffer B, and pressure traces, over a 10-minute interval. This is useful during column equilibration. The chromatogram window is displayed at the bottom of the screen, under the valve control panel (See Figures 1 and 2). Features of the chromatogram window include:

- The time axis is reset automatically at the end of 10 minutes or reset manually by clicking the Clear Traces button
- The chromatogram window can be enlarged by pressing the Resize button.
- A chromatogram trace may be selected for scaling by using the drop-down menus on the upper right and left of the display
- The Y-axis scale can be changed using the scroll bars on the right or left of the display
- The maximum and minimum axis settings can be changed by pressing Settings on the manual screen toolbar.

1.8 Status Bar

At the bottom of the Manual screen is a status bar that is continually updated with system parameters.

Section 2. Anion Exchange Separation of Protein Standards

The starter kit enables you to learn to use the DuoFlow 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 (catalog #720-0001). 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, 0.5 M NaCl
- Flow rate — 4.00 ml/min
- Sample volume — 50 µl
- UV detection — 0.1 AUFS
- QuadTec detection — 0.1 AUFS (λ = 280 nm), 0.1 AUFS (λ = 260 nm), 1.0 AUFS (λ = 214 nm), and 0.4 AUFS (λ = 304 nm)
- Conductivity — 100 mS/cm

General Procedure

Step 1 — Prepare buffer

Step 2 — Prepare sample

Step 3 — Install the UNO Q1 column

Step 4 — Prime the workstation pumps and equilibrate the column

Step 5 — Write a method

a. Program the instrument Setup

b. Program the method Protocol

c. Load sample into 50 µl loop

d. Select Run

e. Select Start

2.2 Prepare Buffers

During solution preparation, wear appropriate laboratory protective clothing including, eye protection, and gloves. Avoid skin and eye contact with starter kit solutions. In case solutions come in contact with eyes, rinse immediately with plenty of water and get medical advice.

Buffer A

a. Empty the contents of the bottle labeled buffer A into a 500 ml graduated cylinder and add filtered, high-quality water to a 500 ml volume.

b. Place the contents of the graduated cylinder into a 1 L side-arm flask and drop in a stirbar. Cap the side arm flask, place it on a stirplate and connect it to a vacuum source. Degas the buffer for approximately 15 minutes with gentle stirring.

c. When degassing is complete, pour the buffer into a bottle and label it “Buffer A, 25 mM Tris-HCl, pH 8.1”.

Buffer B

Prepare buffer B by following the same procedure for preparation of buffer A. Label the buffer as “Buffer B = 25 mM Tris-HCl, pH 8.1, 0.5 M NaCl”.

Conversion of Maximizer Solutions to Buffers A and B

The starter kit contains solutions for use with the DuoFlow Maximizer or Pathfinder systems. These can be converted to buffer A, 25 mM Tris-HCl, pH 8.1, and buffer B = 25 mM Tris-HCl, pH 8.1, 0.5 M NaCl as follows:

a. Dilute Maximizer solutions A1 and A2 to 500 ml each with filtered water.

b. Combine 150 ml of diluted A1, 100 ml of diluted A2 and the entire contents of solution B2. Dilute the mixture to 500 ml. Check the pH and adjust to pH 8.1, if necessary. Degas the solution and label it as “Buffer B, 25 mM Tris-HCl, pH 8.1, 0.5 M NaCl“.

c. Combine the remaining diluted solutions A1 and A2 with water in a 1:1:2 ratio (i.e., 250 ml each of diluted A1 and A2 with 500 ml water). Check the pH and adjust it to pH 8.1, if necessary. Degas the solution and label it as “Buffer A, 25 mM Tris-HCl, pH 8.1”.

2.3 Prepare Sample

a. Remove the aluminum cap from the anion exchange standard vial. Slowly remove the rubber plug from the vial (the contents may be under vacuum).

b. Add 1.0 ml of prepared buffer A to the vial.

c. Replace the rubber stopper and gently invert the vial to solubilize the protein standards.

2.4 Install the UNO Q1 column

Remove the end caps from the UNO Q1 column. Keeping tubing lengths to a minimum, connect 1/16" tubing from port 4 of the AVR7-3 inject valve to the column inlet. Connect the column outlet to the bottom of the UV flow cell or to the QuadTec flow cell. Secure the column in a vertical position.

2.5 Prime the Pumps and Equilibrate the UNO Q1 Column

Ensure the pumps are stopped and the inject valve is in the purge position. Re-prime and purge pumps A and B as described in Section 1.1 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.

a. Wash the column with 6.5 ml (5 column volumes) of buffer B at 2 ml/min.

b. Equilibrate the column with 13 ml (10 column volumes) of 100% buffer A. The conductivity monitor on the status bar should now read ≤ 3 mS/cm.

2.6 Create a New Method

In the Manual screen, select the Browser icon from the tool bar. In the Browser screen you will enter a user name for your method (refer to page 6-1 of the DuoFlow instruction manual for more information on the Browser screen) according to the following steps:

- Select the Browser icon from the tool bar menu
- Select the New icon from the upper left side of the Browser screen
- Select New from the drop-down menu and enter your user name in the dialog box.
- Click on the Project icon for your user name
- Select New and New Method. Enter your method name (or use default Method 1)
- Click OK to proceed to the instrument/devices Setup screen

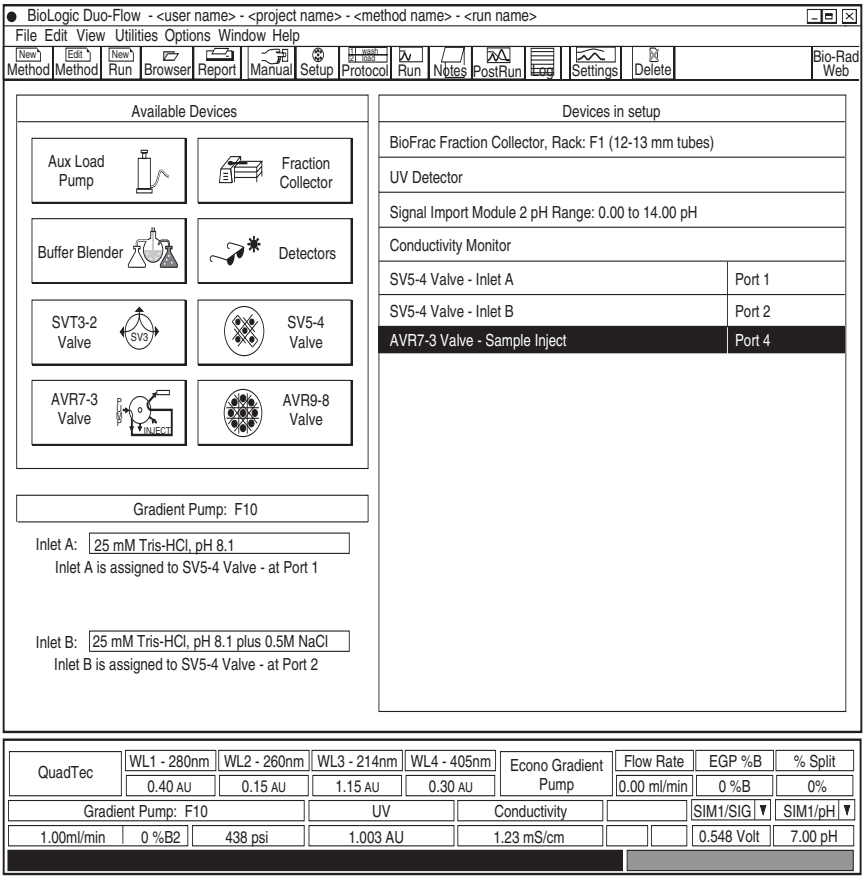


Fig. 3. Setup editor

Program the Instrument Setup

In the Setup screen select the instruments and devices to be used for the Starter Kit method. The icons grouped on the left side of the screen (refer to Figure 3, Available Devices) show all the instruments and devices that can be connected to the BioLogic DuoFlow systems.

The list of devices in the right box (Devices in Setup) identifies those devices selected for use with a specific method. The initial default Devices in Setup are a UV detector, conductivity monitor, and an AVR7-3 inject valve. These come

standard with the BioLogic DuoFlow system. The DuoFlow QuadTec system includes a QuadTec UV/Vis detector in place of a UV detector.

a. 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 BioFrac. Click on BioFrac and click the OK button. You will now see BioFrac fraction collector in the Devices in Setup box. The F1 Rack (12–13 mm tubes) is automatically selected.

b. If you are using a QuadTec UV/Vis detector, click on the Detectors button in the Available Devices box. A dialog box will appear asking you to choose a detector. Select QuadTec and check each of the four wavelength boxes. Enter the wavelengths: (1) 280 nm, (2) 260 nm, (3) 214 nm, and (4) 405 nm. Press OK.

c. In the Gradient Pump section of the setup screen enter your buffer names. In the buffer A field, type in 25 mM Tris-HCl, pH 8.1. In the buffer B field type in 25 mM Tris-HCl + 0.5 M NaCl, pH 8.1.

d. The Setup is now complete. To save the device setup, choose Save Setup under the File menu and enter a name for your Setup.

e. You are now ready to program the separation steps for your method. To program your method, press the Protocol icon on the tool bar.

Program the Method Protocol

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

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

- From the left side of the screen, press the fraction collection icon. In the pop-up window that appears, choose Collect All with a fraction size of 2.00 ml and a delay of 0.0. Make sure the correct rack type is displayed.
- Program the remaining steps using the Add Step icons from the left side of the screen.

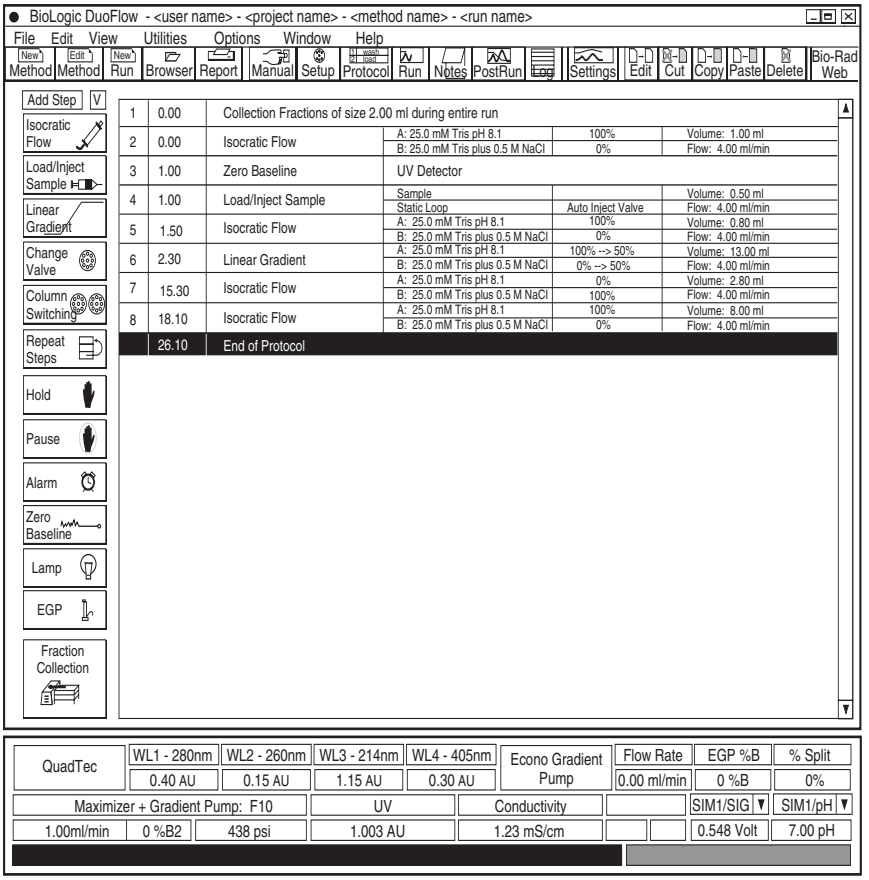


Fig. 4. Protocol screen

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-HCl, pH 8.1, 0% 25 mM Tris-HCl, 0.5 M NaCl, pH 8.1 at 4.00 ml/min for 1.0 ml
3.	1.0	Zero Baseline to set UV baseline to 0.0. Select either UV detector or QuadTec detector.
4.	1.0	Load inject sample, static loop: Inject 0.5 ml sample at 4.00 ml/min. You will be injecting the loop size of 50 µl.
5.	1.5	Isocratic flow with 100% 25 mM Tris-HCl, pH 8.1, 0% 25 mM Tris-HCl, 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

c. When you have finished programming the method, press the toolbar button RUN. A dialog box will ask you to name the run. Accept the default Run 1 and click the OK button. You will now see the Run screen (see Figure 5).

The Run Screen

a. The toolbar buttons on the left side of the screen enable you to check that the screen display ranges for UV (see page 8), QuadTec UV/Vis and conductivity are correctly set and that the gradient pump pressure limits are appropriate (700 psi high and 20 psi low limit), for the UNO Q1 column.

b. If you have been equilibrating the column while writing the method, you will notice that the Status Bar is displaying the flow rate and values for UV, QuadTec UV/Vis, and conductivity detectors. If necessary, you may wish to

zero the UV or QuadTec UV/Vis trace by clicking on the Zero baseline button in the appropriate box. This button may be selected at any time.

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

d. To enlarge the view select the Resize button to the right of the chromatogram display.

Start the Run

a. Ensure that sufficient tubes are in the fraction collector rack (approximately 14). The drophead will automatically move to tube 1 when the run is started.

b. Ensure 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.

c. Ensure that the 50 µl sample loop is connected to ports 3 and 6 of the inject valve. Completely fill the loop with protein standard via port 2 using the syringe and needle provided. Do not remove the syringe from the injection port after filling the loop or the sample will siphon to waste.

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

e. When the run is finished, the pumps automatically stop and a “Run Finished” message appears in the bottom right of the status bar.

f. Figures 5 and 6 show typical run screens and chromatograms for this separation using the UV or QuadTec UV/Vis detectors.

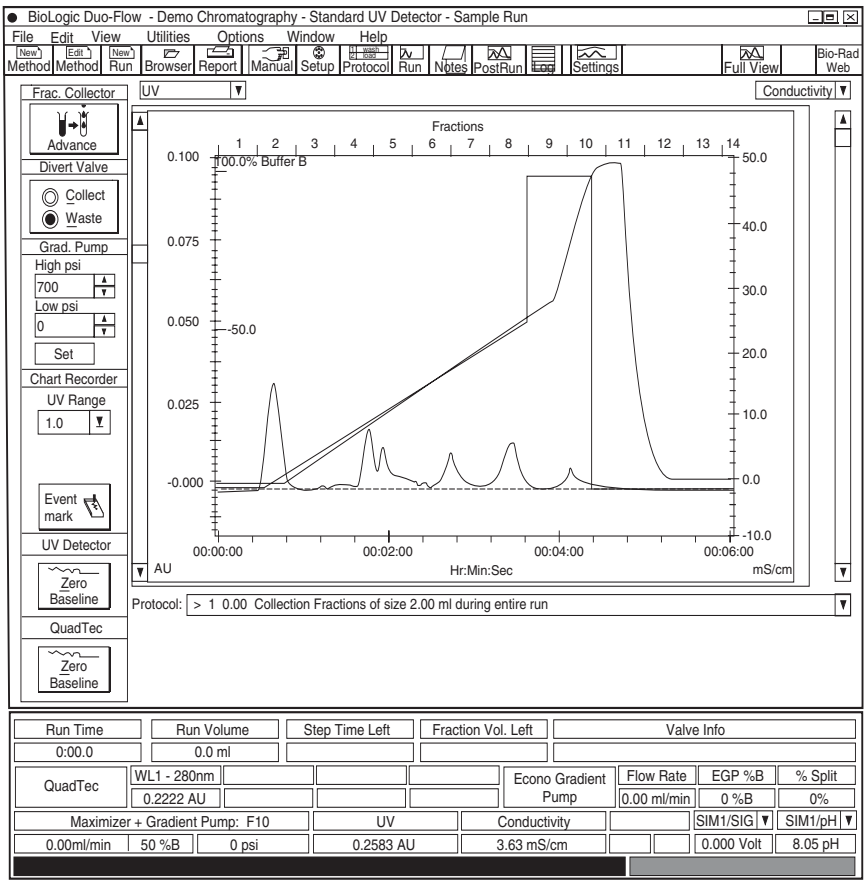


Fig. 5. Run Screen (UV detector and conductivity traces)

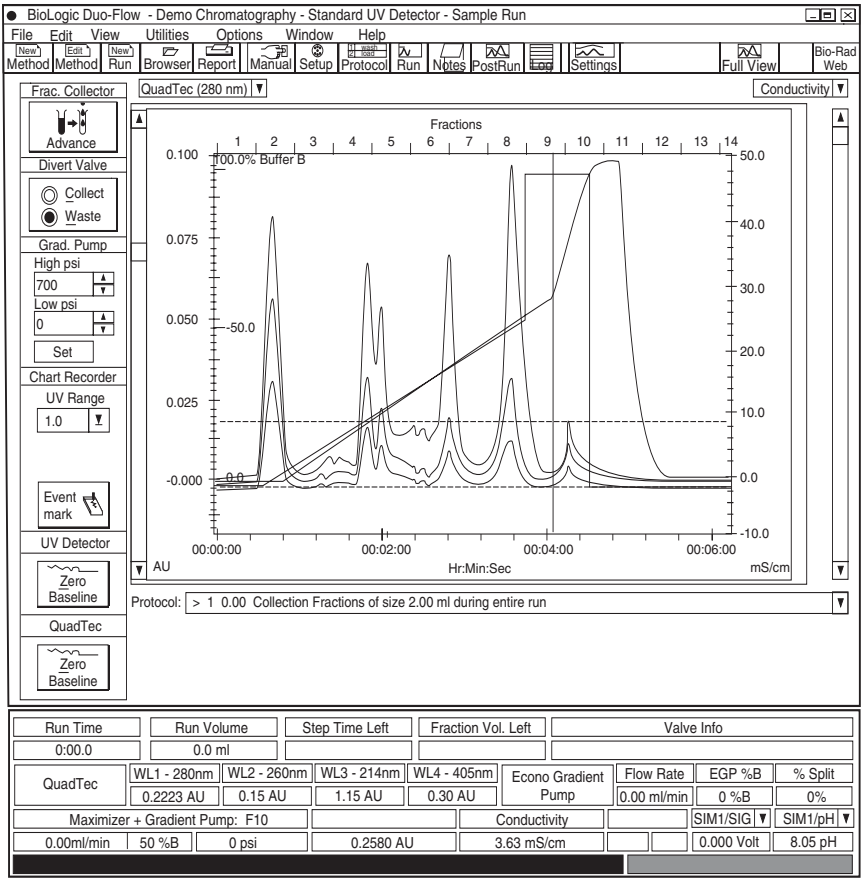


Fig. 6. Run screen QuadTec UV/Vis traces

II. DuoFlow Maximizer and Pathfinder Systems

Section 3. System Preparation

When the DuoFlow Maximizer or Pathfinder system is turned on, the Manual screen is displayed in either Buffer Blending (Figure 7) or Non-Buffer Blending mode (Figure 8). This screen displays instrument control panels that provide direct control of the pumps, valves, fraction collector, UV detector, QuadTec UV/Vis detector, and Econo gradient pump. The arrow in the upper right corner of the detector control panel toggles between the UV and QuadTec detector control panels. The button in the upper right hand corner of the valve control panel toggles between the workstation and Maximizer valve control panels.

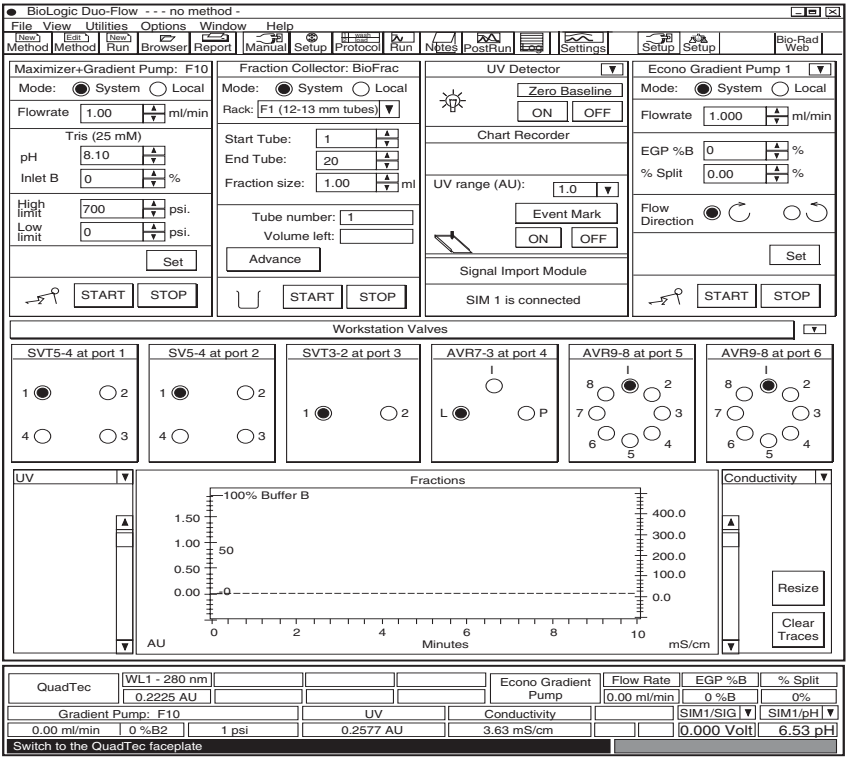


Fig. 7. Manual screen (Buffer Blending mode)

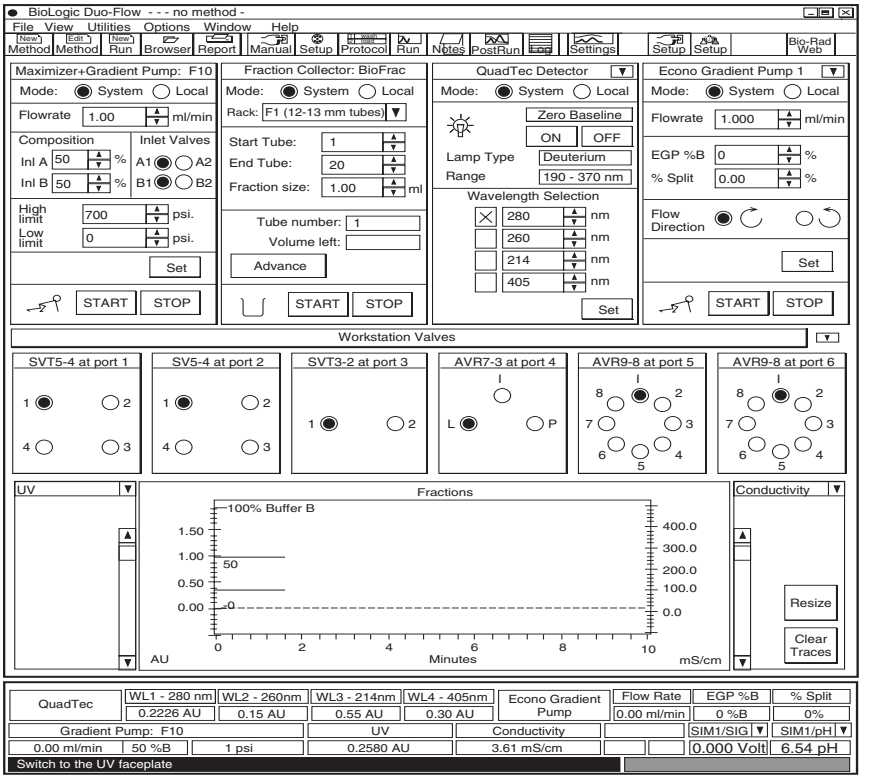


Fig. 8. Manual Screen (Non-Buffer Blending mode)

3.1 Prime the Workstation Pumps

a. Immerse Maximizer inlet lines A1, A2, B1, and B2 in a container of HPLC grade (filtered, degassed) or other high-quality water.

b. From the Manual screen place the Maximizer in Local mode and use the Valve Port Select button, under the A1/A2 Maximizer valve inlet, to select inlet port A1.

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

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

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

f. Use the Valve Port Select button on the Maximizer to select solution A2. Gently withdraw the syringe plunger to draw water into the pump head.

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

h. Tighten the priming port by turning it clockwise.

i. Repeat this priming procedure for pump B and inlets B1 and B2.

3.2 Move the AVR7-3 Inject Valve to the Purge Position

Prior to purging the pumps at 10 ml/min it is essential to place the AVR7-3 valve in the purge position. This directs the flow to waste and not to the columns and detector.

To change the position of the AVR7-3 inject valve, select P from the Manual screen valve control panel for the AVR7-3 valve. Select the workstation or Maximizer valve control panel that displays the AVR7-3 inject valve. For example, if you have connected the AVR7-3 inject valve into port 10, you will see a valve box designated AVR7-3 at port 10 of the Maximizer valve control panel. The three 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.

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.

3.3 Purge the Workstation Pumps

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

b. Select solutions A2 and B2 from the Valve Port Select buttons on the Maximizer faceplate.

c. Press the Purge buttons A then B on the front of the workstation. The workstation pumps will run at 10 ml/min and the indicator light will flash green.

d. Run each pump for 2 minutes and then press the Valve Port Select buttons on the Maximizer faceplate to select solutions A1 and B1. Run each pump for 2 minutes more and then press the Purge buttons to stop the pumps.

3.4 Manual Control of the Pumps

The workstation pump parameters can be set from the Manual screen Maximizer+Gradient Pump control panel either by clicking in the appropriate field and entering a value from the keyboard or by using the arrows. The appearance of the these control panels depends on whether the instrument is in Buffer Blending or Non-Buffer Blending mode. Figures 7 and 8 show examples of the respective Buffer Blending and Non-Buffer Blending control panels. From these control panels you can set the flow rate, pressure limits, and buffer composition. You can switch between these two panels by pressing 

in the toolbar and checking or unchecking the “Use Buffer Blending” box on the Maximizer Buffer Blending Setup screen.

To start the pump, click the Start button. Note that the running man icon will start running. 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. If you are using an UNO Q1 column, set the high limit to 700 psi and the low limit to 20 psi.

3.5 Flush the System Through to the Fraction Collector

With the gradient pumps stopped, move the AVR7-3 inject valve back to position L (Load) by clicking L (AVR7-3) on the Manual screen.

From the gradient pump control panel on the Manual screen, set the pump flow rate at 1.0 ml/min and start the pump. Water will flow through the UV or QuadTec and conductivity flow cells to the fraction collector, as described below.

BioFrac Fraction Collector

The BioFrac fraction collector has two operating modes:

- System—Controlled by the BioLogic DuoFlow system
- Local—Controlled from its own faceplate in stand-alone mode

Ensure that the System button is selected.

When in System mode, the fraction collector control panel on the Manual screen will show fields for Rack type, Start tube, End tube, Fraction size, Tube number, Volume left, a toggle button for Start and Stop, and a button for Advance (see Figures 7 and 8).

3.6 Turn on the UV Lamp

DuoFlow UV Detector

a. The UV lamp automatically turns on when you turn on power to the workstation. The UV lamp can be turned on and off by clicking the On and Off buttons from the UV detector control panel on the Manual screen (see Figure 7). Check that the lamp is on; the mercury lamp requires approximately 30 minutes to warm up.

b. Select the Zero Baseline button to zero the UV signal. The Status bar along the bottom of the screen provides AU output for the detector. Ensure that it goes to zero when you select the zero baseline option.

QuadTec UV/Vis Detector

a. The QuadTec detector should be powered On before starting the BioLogic software. If the QuadTec detector is not powered up, exit the software, power up the QuadTec detector, and restart the software. When connection is completed, “SLAVE” appears in the upper left corner of the QuadTec faceplate.

b. From the QuadTec detector control panel on the Manual screen (Figure 7), set the four wavelengths of the QuadTec detector to 280, 260, 214, and 405 nm. Select Set. The active wavelength will appear in the lower screen status bar.

c Click the Zero Baseline button to zero the four UV/Vis signals. The Status bar along the bottom of the screen provides AU output for the detector. Ensure that it goes to zero when you select the zero baseline option.

3.7 pH Electrode Calibration

Prior to starting a run the pH electrode should be calibrated as follows:

a. Remove the electrode from the flow cell and rinse it with deionized water.

b. Place the electrode in pH 7 standard buffer.

c. From the Utilities drop-down menu, select “pH probe calibration”.

d. Enter the temperature and reference pH (at the current temperature) for the first buffer. Press Set. The temperature can be read from the Display Screen on the Maximizer.

e. When the pH reading has stabilized, press OK.

f. Rinse the electrode with deionized water.

g. Place the electrode in the pH 10 standard buffer.

h. Enter the reference pH for the second buffer at the current temperature. Press Set.

i. When the pH reading has stabilized, press OK.

3.8 Manual Screen Chromatogram Window

A feature of the Manual screen is its ability to display up to eight traces of a chromatogram, including UV/Vis, pH, conductivity, % Buffer B, and pressure traces over a 10-minute interval. This is useful during column equilibration. The chromatogram window is displayed at the bottom of the screen, under the valve control panel (See Figures 7 and 8). Features of the chromatogram window include:

- The time axis is reset automatically at the end of 10 minutes or may be reset manually by clicking the Clear Traces button
- The chromatogram window can be enlarged by pressing the Resize button
- A chromatogram trace may be selected for scaling by using the drop-down menus on the upper right and left of the display
- The Y-axis scale can be changed using the scroll bars on the right or left of the display
- The maximum and minimum axis settings can be changed by pressing Settings on the manual screen toolbar

3.9 Status Bar

At the bottom of the Manual screen is a status bar that is continually updated with system parameters.

Section 4. Anion Exchange Separation of Protein Standards

The starter kit enables you to learn to use the DuoFlow Maximizer or Pathfinder systems by programming and running 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 (catalog #720-0001). 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 A1 — 50 mM Tris-HCl
- Buffer A2 — 50 mM Tris base
- Solution B1 — deionized water
- Solution B2 — 2.0 M NaCl
- Flow rate — 4.00 ml/min
- Sample volume — 50 µl
- UV detection — 0.1 AUFS
- QuadTec detection — 0.1 AUFS (λ = 280 nm), 0.1 AUFS (λ = 260 nm), 1.0 AUFS (λ = 214nm), and 0.4 AUFS (λ = 304 nm)
- Conductivity — 100 mS/cm
- pH — pH 7.1 to pH 9.1

General Procedure

Step 1 — Prepare solutions A_1, A_2, B_1, and B_2

Step 2 — Prepare sample

Step 3 — Install the UNO Q1 column

Step 4 Prime the workstation pumps and inlet valves, and equilibrate the column

Step 5 Write a method

a. Program in the instrument Setup

b. Program the method Protocol

c. Load sample into 50 µl loop

d. Select Run

e. Select Start

4.2 Prepare Solutions

During solution preparation, wear appropriate laboratory protective clothing, including eye protection and gloves. Avoid skin and eye contact with starter kit solutions. In case solutions come in contact with eyes, rinse immediately with plenty of water and get medical advice.

Note: Solutions A, and A_2 are diluted to **500 ml**, solution B_2 is diluted to **125 ml**.

Solution A1

a. Empty the contents of the bottle labeled Solution A1 into a 500 ml graduated cylinder and add filtered, high-quality water to a 500 ml volume.

b. Place the contents of the graduated cylinder into a 1 L side-arm flask and drop in a stirbar. Cap the side arm flask, place it on a stirplate and connect it to a vacuum source. Degas the solution for approximately 15 minutes with gentle stirring.

c. When degassing is complete, pour the buffer into a bottle and label as "Solution A1 50 mM Tris-HCl".

Solution A2

a. Empty the contents of the bottle labeled Solution A2 into a 500 ml graduated cylinder and add filtered, high-quality water to 500 ml. Caution: pH of Solution A2 is approximately 10.5.

b. Place the contents of the graduated cylinder into a 1 L side-arm flask and drop in a stirbar. Cap the side arm flask, place it on a stirplate and connect it to a vacuum source. Degas the solution for approximately 15 minutes with gentle stirring.

c. When degassing is complete, pour the buffer into a bottle and label “Solution A2, 50 mM Tris base”.

Solution B1

Place 1 L of water into a 1 L side-arm flask and drop in a stirbar. Cap the side arm flask, place it on a stirplate and connect it to a vacuum source. Degas the solution for approximately 15 minutes with gentle stirring. Label solution as “Solution B1, water”.

Solution B2

a. Empty the contents of the bottle labeled Solution B2 into a 200 ml graduated cylinder and add filtered, high-quality water to **125 ml**. If you mistakenly dilute solution B_2 to 500 ml, add 43.8 g NaCl, stir, and degas until dissolved.

b. Place the contents of the graduated cylinder into a 1 L side-arm flask and drop in a stirbar. Cap the side-arm flask, place it on a stirplate, and connect it to a vacuum source. Degas the solution for approximately 15 minutes with gentle stirring.

c. When degassing is complete, pour the buffer into a bottle and label it as “Solution B2, 2 M NaCl”.

4.3 Prepare Sample

a. Using the fraction collector, collect 1 ml of pH 8.1 Tris, 0% B into a tube. Alternatively, mix 0.25 ml of Solution A1, 0.25 ml of Solution A2, and 0.5 ml of water in an Eppendorf tube.

b. Remove the aluminum cap from the anion exchange standard vial. Slowly remove the rubber plug from the vial (the contents may be under vacuum).

c. Add 1.0 ml of the buffer from step (a) to the vial.

d. Replace the rubber stopper and gently invert the vial to solubilize the protein standards.

4.4 Install 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 AVR7-3 inject valve to the column inlet. Connect the column outlet to the bottom of the UV flow cell or QuadTec flow cell. Secure the column in a vertical position.

4.5 Prime the Pumps and Equilibrate the UNO Q1 Column

Ensure the gradient pumps are stopped and the inject valve is in the purge position. Place the tubing from inlets A1, A2, B1 and B2 into solutions A1 (Tris-HCl), A2 (Tris base), B1 (water), and B2 (NaCl), respectively. Re-prime and purge the pumps and inlets A1, A2, B1, and B2 as described in Section 3.1 of this manual.

Set the inject valve to position L (Load). Set the flow rate to 2.0 ml/min. Set the UV (280 nm) range to 0.1 AUFS and the conductivity range to 100 mS/cm.

Set the buffer recipe by pressing 

on the toolbar and choosing Tris (25 mM) as your buffer. Press OK. On the manual screen set the pH to 8.10 and the Salt Molarity %B to 0.0.

Change the pH scale by pressing the Settings button on the toolbar. Set the minimum pH to 7.1 and the maximum pH to 9.1 in SIM1/pH.

Applying a 1 or 2-point pH correction (optional).

The Maximizer has been designed to prepare buffers accurately and reproducibly at a user-defined pH and salt composition. The pH accuracy, however, depends on how close the selected pH is to the buffer systems pKa. In situations where high pH accuracy is required, you should apply a 1 or 2-point pH correction as described below.

Single Point Correction (best for isocratic applications)

a. In the manual screen set the pH to 8.1, the salt composition to 0 %B (or to the desired %B) and the flow rate to 2.0 ml/min.

b. Take the column out of line if it has been connected and start the pump.

c. When the pH has stabilized, read the pH from the status bar or, alternatively, collect the effluent and measure the pH using a high quality Tris compatible pH probe.

d. From the buffer blending setup screen, place a check in the Use pH Correction box (leave the Use Two Point Correction box unchecked).

e. Set the desired pH to 8.1 and the Observed at 0 % pH, to the pH measured in step (c).

f. Press OK.

Two-Point Correction (best for gradient applications)

a. In the manual screen, set the pH to 8.1, the salt composition to 0 %B and the flow rate to 2.0 ml/min.

b. Take the column out of line, if it has been connected, and start the pump.

c. When the pH has stabilized, read the pH from the status bar or, alternatively, collect the effluent and measure the pH using a high quality Tris compatible pH probe.

d. Change %B to 100 % (or to the maximum %B that will be used for the experiment).

e. When the pH reading has stabilized, read the pH from the status bar or, alternatively, collect the effluent and measure the pH using a high quality Tris compatible pH probe.

f. Set the %B back to 0% and re-equilibrate the system with the low salt buffer.

g. From the buffer blending setup screen, place a check in the Use pH Correction box.

h. Set the desired pH to 8.1 and the Observed at 0 %B pH to the pH measured in step (c).

i. Place a check in the Use Two Point Correction box and set %B to the value used in step (d)).

j. Set the Observed at %B pH to the pH measured in step (e).

k. Press OK.

Column Equilibration.

a. Connect the UNO Q1 column.

b. Wash the column with 6.5 ml (5 column volumes) of pH 8.10 Tris (100% B) at 2 ml/min.

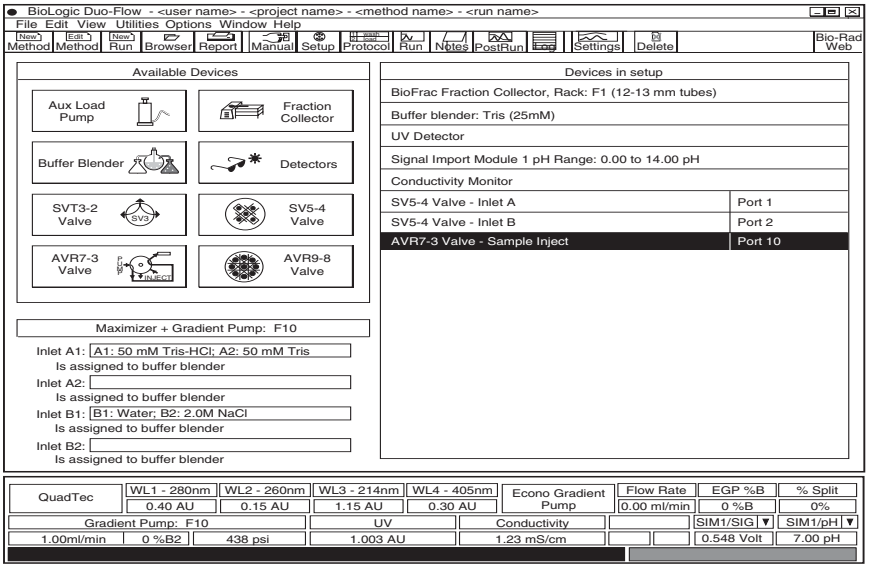


Fig. 9. Setup editor

c. Equilibrate the column with 13 ml (10 column volumes) of pH 8.10 Tris (0% B). When finished, the conductivity monitor on the status bar should read ≤ 3 mS/cm.

4.6 Create a New Method

In the Manual screen select the Browser icon from the tool bar. In the Browser screen you will enter a user name and name for your method (refer to page 6-1 of the DuoFlow instruction manual for more information on the Browser screen) according to the following steps:

- Select the Browser icon from the tool bar menu
- Select the New icon from the upper left side of the Browser screen
- Select New from the drop-down menu and enter your user name in the dialog box
- Click on the Project icon for your user name
- Select New and New Method. Enter your method name (or use default Method 1)
- Click OK to proceed to the instrument/devices Setup screen

Program the Instrument Setup

In the Setup screen, select the instruments and devices to be used for the Starter Kit method. The icons grouped on the left hand side of the screen (refer to Figure 3, Available Devices), show all the instruments and devices that can be connected to the BioLogic DuoFlow systems.

The list of devices in the right box (Devices in Setup) identifies those devices selected for use with a specific method. The initial default Devices in Setup are a UV detector, a conductivity monitor, and an AVR7-3 inject valve, as these come as standard with the BioLogic DuoFlow system. The DuoFlow QuadTec systems includes a QuadTec UV/Vis detector in place of a UV detector.

a. Click on the Buffer Blending button in the Available Devices box. A dialog box will appear. Choose Tris (25) mM from the buffer list and select OK. If desired, enter the 1 or 2-point pH correction measured in Section 4.5.

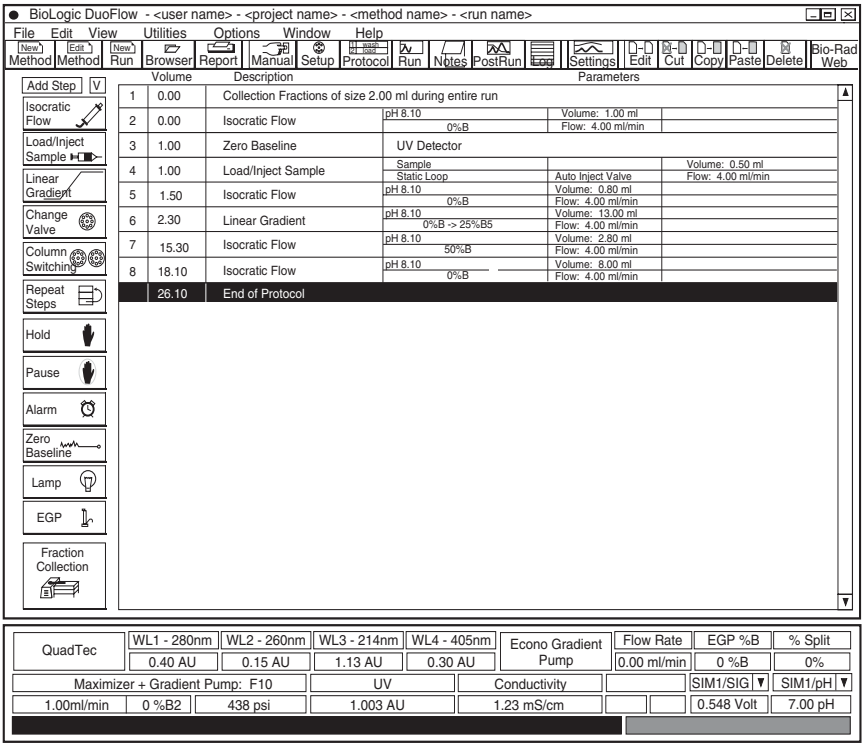


Fig. 10. Protocol editor

b. 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 BioFrac. Click on BioFrac and click the OK button. You will now see the BioFrac fraction collector in the Devices in Setup box. The F1 Rack (12–13 mm tubes) is automatically selected.

c. If you are using a QuadTec UV/Vis detector, click on the Detectors button in the Available Devices box. A dialog box will appear asking you to choose a detector. Select QuadTec and check each of the four wavelengths boxes. Enter the wavelengths: (1) 280 nm, (2) 260 nm, (3) 214 nm, and (4) 405 nm. Press OK.

d. The Setup is now complete. To save the device setup, choose Save Setup under the File menu and enter a name for your Setup.

e. You are now ready to program the separation steps for your method. To program your method, press the Protocol icon on the tool bar.

Program the Method Protocol

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

b. Program the separation method listed below and in Figure 10.

- From the left side of the screen, press the fraction collection icon. In the pop-up window that appears, choose Collect All with a fraction size of 2.00 ml and a delay of 0.0. Make sure the correct rack type is displayed.
- 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	Isocratic flow with 25 mM Tris-HCl, pH 8.1, 0% 1.0 M NaCl, at 4.00 ml/min for 1.0 ml
3.	1.0	Zero Baseline to set UV baseline to 0.0. Select either UV detector or QuadTec
4.	1.0	Load Inject Sample Static loop: Inject 0.5 ml sample using 25 mM Tris, pH 8.1, 0% 1.0 M NaCl at 4.00 ml/min
5.	1.5	Isocratic flow with 25 mM Tris, pH 8.1, 0% 1.0 M NaCl at 4.00 ml/min for 0.8 ml
6.	2.3	Linear gradient with 0% to 25% 1.0 M NaCl at 4.00 ml/min for 13.0 ml
7.	15.3	Isocratic flow at 25 mM Tris, pH 8.1 and 50% 1.0 M NaCl at 4.00 ml/min for 2.8 ml
8.	18.1	Isocratic flow with 25 mM Tris, pH 8.1, 0% 1.0 M NaCl at 4.00 ml/min for 8.0 ml
9.	26.1	End of protocol

c. When you have finished programming the method protocol, press the toolbar button RUN. A dialog box will ask you to name the run. Accept the default Run 1 and click the OK button. You will now see the Run screen (see Figures 11 and 12).

The Run Screen

a. The toolbar buttons on the left side of the screen enable you to check that the screen display ranges for UV, QuadTec UV/Vis, pH, and conductivity are correctly set (see page 24) and that the workstation pump pressure limits are appropriate (700 psi high and 20 psi low limit) for the UNO Q1 column.

b. If you have been equilibrating the column while writing the method, you will notice that the Status Bar is displaying the flow rate and values for UV, QuadTec UV/Vis, and conductivity. If necessary, you may zero the UV or QuadTec UV/Vis trace by clicking on the Zero baseline button on the appropriate box. This button may be selected at any time.

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

d. To enlarge the view select the Resize button to the right of the chromatogram display.

Start the Run

a. Ensure that sufficient tubes are in the fraction collector rack (approximately 14). The drophead will automatically move to tube 1 when the Run is started.

b. Ensure 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.

c. Ensure that the 50 µl sample loop is connected to ports 3 and 6 of the inject valve. Completely fill the loop with 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.

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

e. When the run is finished, the pumps automatically stop and a “Run Finished” message appears in the bottom right of the status bar.

f. Figures 12 and 13 show typical run screens and chromatograms for this separation using the UV detector or QuadTec UV/Vis detector.

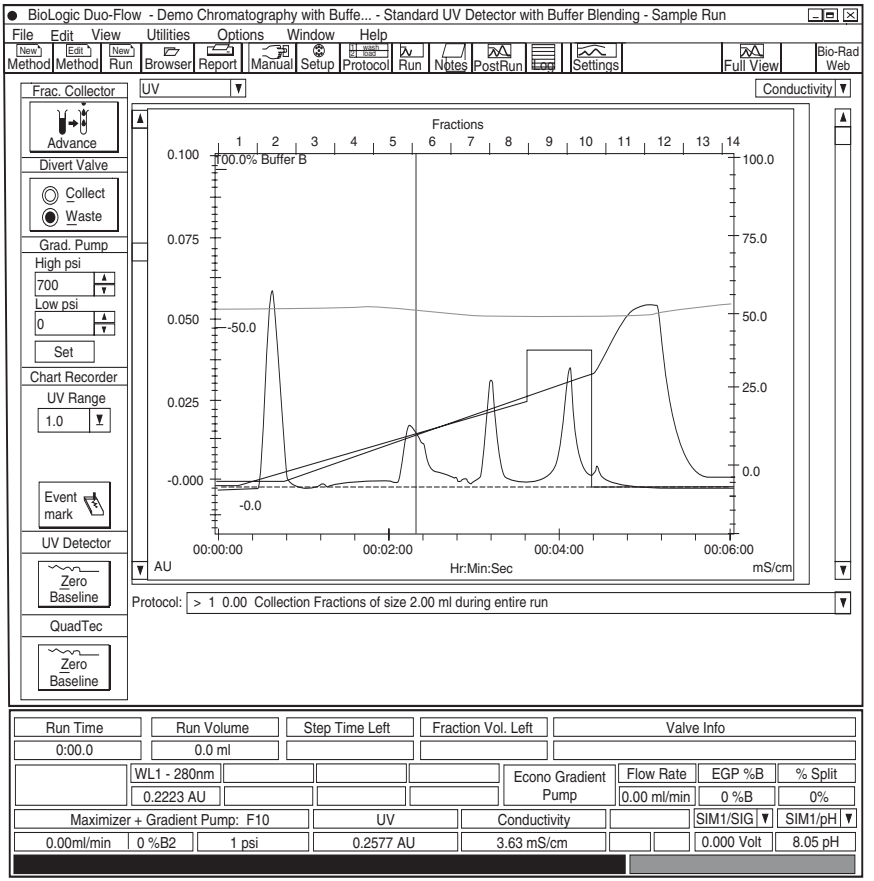


Fig. 11. Run screen (UV detector and conductivity traces)

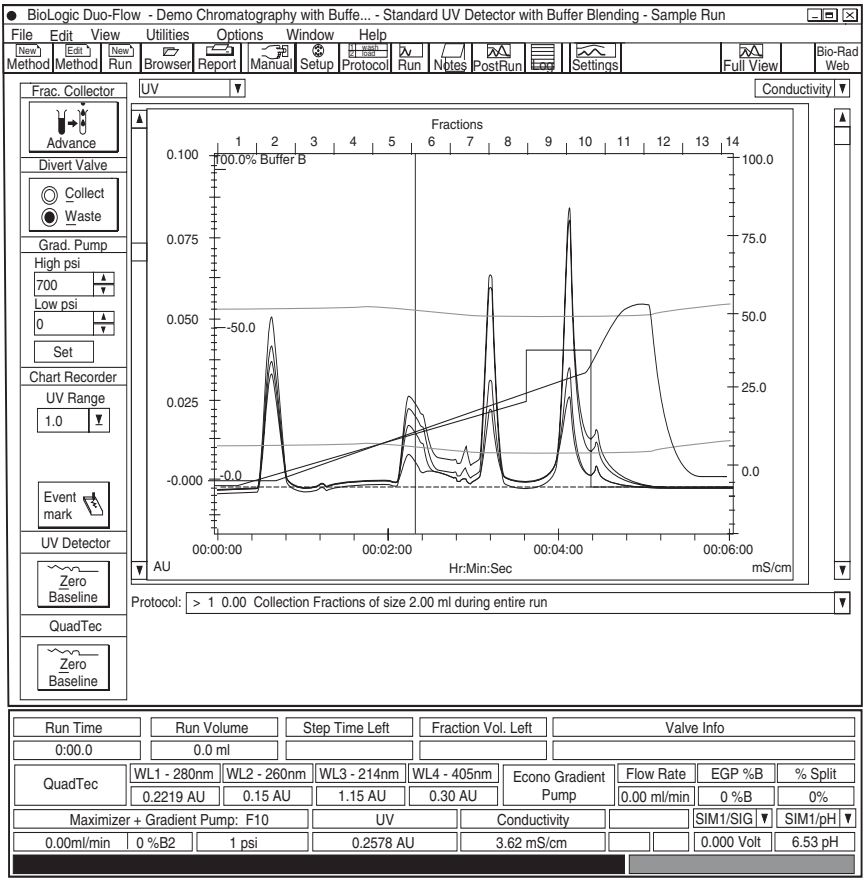


Fig. 12. Run screen (QuadTec UV/Vis detector traces)

Section 5. Ordering Information

Catalog #	Description
760-0135	Starter Kit
760-0047	BioLogic DuoFlow Standard System, 100/120 V, includes Dell controller and monitor, USB Bitbus communicator, F10 workstation, MX-1 mixer, 3-tray rack, AVR7-3 sample inject valve, fittings kit, UV detector with 5 mm flow cell and 254/280 nm filters, conductivity monitor, BioFrac fraction collector with diverter valve and two F1 racks, starter kit, UNO Q1 column and instructions
760-2200	BioLogic Maximizer Kit, 110/120 V, includes Maximizer base unit, pH electrode and flow cell, Maximizer mixer, starter kit, tubing kit, system cable 30, US power cord
760-1300	BioLogic QuadTec Detector Kit, includes QuadTec detector with 3 mm PEEK flow cell, Instrument control module (ICM, System cable 25, 26, and 17 (QuadTec RS-232, ICM power, and bus communication), US power cord, instructions
741-0002	BioFrac Fraction Collector, includes a 110 V power cord, rack F1(2), Econo system cable #15, and fittings kit
	Collection Tubes*
223-9500	1.5 ml Capless Micro Test Tubes, polypropylene, natural, 500/box
223-9750	13 x 100 mm Clear Polystyrene Test Tubes, 1,000/box
223-9750	13 x 100 mm Natural Polypropylene Test Tubes, 1,000/box

* Additional tubes sizes are available from Bio-Rad. Contact your local Bio-Rad representative for a liquid handling catalog.

For technical service, call your local Bio-Rad office, or in the U.S., call
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