Econo System Starter Kit

Instruction Manual

Instructions for the First Time User of the Econo System and Automated Econo System

Catalog Number 731-8182

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Introduction

This manual will guide you through the installation of your new Econo System. In addition, this manual will show you how to perform an actual protein separation to fully demonstrate the simplicity and excellent performance of your new Econo System.

Starter Kit Components

This Starter Kit contains the following items:

One Econo-Pac® Q anion exchange cartridge, 1 ml

Buffer A, a 10x concentrate of Tris buffer, 50 ml

Buffer B, a 10x concentrate of Tris buffer and NaCl, 50 ml

One vial of Anion Exchange Protein Standards

One disposable sample injection syringe

Instruction Manual

For the first time user, set-up and plumbing of the Econo System will require approximately 3 to 4 hours. The chromatographic separations performed in this kit require approximately 30 minutes. The starter kit provides enough buffer and sample to perform a minimum of three separations.

Other Materials You Will Need

- Deionized water, 1 L
- One 500 ml graduated cylinder
- One 1 L sidearm flask
- Stir bar and stir plate
- Vacuum source, either house vacuum, mechanical pump, or faucet-mounted aspirator
- Two 500 ml bottles
- 500 ml of 0.1 N NaOH solution (2 g NaOH / 500 ml)
- 30 collection tubes, 13 x 100 mm
- Scissors or blade to cut tubing

If you have a standard Econo System, proceed with Part I on page 1. If you have an Automated Econo System, we recommend that you also begin with Part I on page 1. This will allow you to become familiar with the basic programming scheme prior to learning how to use the more advanced automated features. After completing Part I, continue with Part II on page 16.

Part I instructions assume that you have a complete standard Econo System, which includes:

- **Econo Pump**
- **Econo UV Monitor**
- **Econo System Controller**
- **Model 2110 Fraction Collector**
- **Single Pen Econo Recorder**
- **Econo System Rack**

Part II instructions assume that you have a complete Automated Econo System, which includes:

- **Econo Pump**
- **Econo UV Monitor**
- **Econo System Controller**
- **Model 2110 Fraction Collector**
- **Econo System Rack**
- **Econo Buffer Selector**
- **Econo Gradient Monitor**
- **Dual Pen Econo Recorder**
- **Econo System Organizer**

Table of Contents

Part I Standard Econo System

Part II Automated Econo System

Section 1
Buffer Preparation

Buffer A

1. Empty the contents of the bottle labeled “buffer A” into a 500 ml graduated cylinder.
2. Add deionized water to 500 ml volume.
3. 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.
4. Degas the buffer under vacuum while stirring. Degas the buffer for approximately 15 minutes.
5. When degassing is complete, pour the buffer into a bottle and label the bottle "buffer A".

Buffer B

Prepare buffer B as described above and label the bottle "buffer B".

While waiting for the buffers to degas, proceed with Section 2, Setting Up the System.

Section 2
Setting up the System

Remove all of the Econo System components from the shipping containers. As you unpack each component, verify that all accessories listed on the Packing List in each container are present.

The Econo System can be set up in a variety of locations, including 4 °C cold rooms or cold boxes. Bench space of approximately 120 cm x 50 cm (W x D) is required.

To set up the Econo System:

1. Place the system controller on the bench. If you have an Automated Econo System, place the system organizer on the bench and place the system controller on top of it. The organizer should be positioned with the metal tabs (on the underside) at the rear.
2. Position and connect the pump and UV monitor to the system controller, using the ribbon cables located on top of the controller, as illustrated in Figure 1.

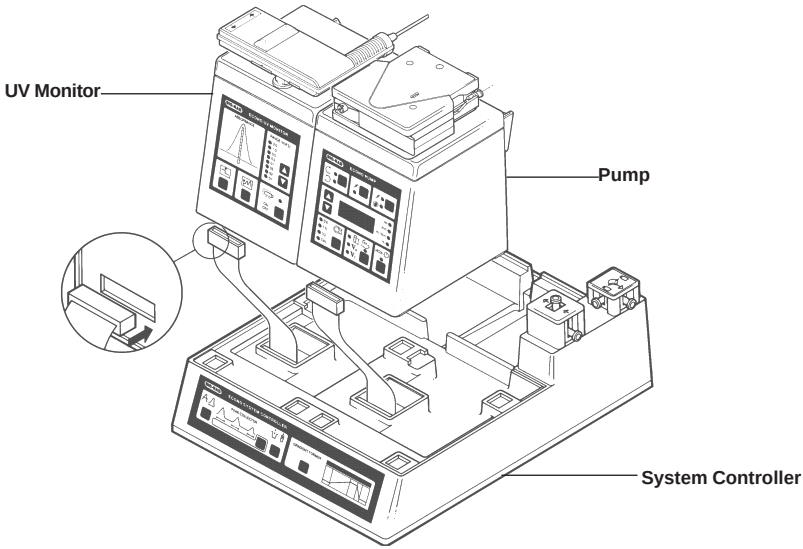


Fig. 1. Connection of the pump and UV monitor to the system controller.

Fig. 2. Positioning the Standard Econo System.

3. Position the chart recorder, rack and fraction collector as illustrated in Figure 2. Connect the chart recorder and fraction collector to the system controller and to power outlets as illustrated in Figure 3. The solvent reservoir included with the system controller sits in the depression on top of the system controller (behind the pump and UV monitor)
4. Attach the diverter valve to the rack and connect it to the rear panel of the system controller (see Figure 3).
5. Attach power cords to the rear panels of the pump and monitor (Figure 3).
6. Place the solvent reservoir in the depression on top of the system controller.

Powering Up

Turn the power on for all components. Power switches are located on the bottom panels of the pump and UV monitor and on top of either single or dual pen chart recorder (the pen switch).

Section 3
Plumbing, Pump Platen Adjustment and Purging

System Plumbing

The Econo System includes enough silicone and Norprene® tubing and luer fittings to completely plumb the system. Tubing can be cut to desired lengths with scissors and luer fittings should be attached to the ends of the tubing for connection of each system component. There are three sizes of luer fittings which correspond to 0.8 mm, 1.6 mm, and 3.2 mm ID sized tubing. It is important to use the correct sized fitting for the diameter of tubing used. If there is firm resistance when pushing the tubing over the barb of the fitting it is likely to be the correct size.

1. To plumb the pump, use the pre-cut 1.6 mm ID. Norprene tubing included with the pump. Slip an orange lock ring over each end of the pre-cut tubing with the bevel pointed away from the end, as illustrated in Figure 4. Insert a barbed female luer fitting into each end of the pre-cut tubing until the tubing reaches the flange. Slide the lock ring towards the luer fitting and push it snugly against the fitting to lock the tubing.

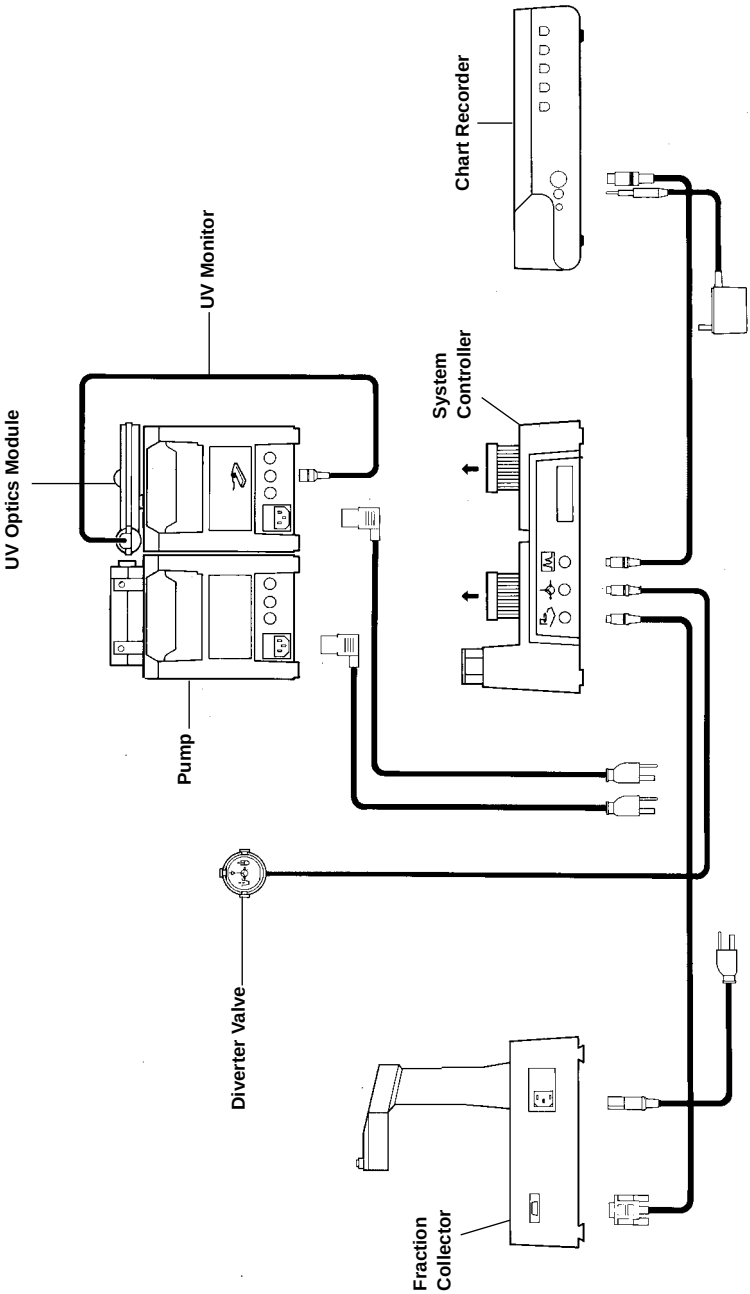


Fig. 3. Electrical connections of the standard Econo System (back panel view of system components).

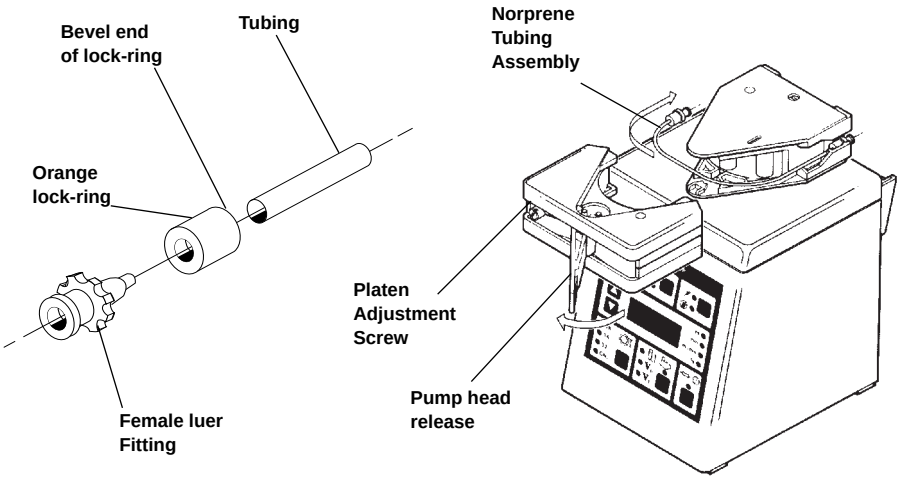


Fig. 4. Pump head and pump tubing assembly.

2. Attach the MV-6 manual injection valve to the rack as illustrated in Figure 2. Connect tubing from the MV-6 injector valve to the pump and waste container as illustrated in Figure 6.

3. To create a 1.5 ml sample loop, cut 72 cm of the 1.6 mm ID tubing and connect it to the MV-6 valve, as illustrated in Figure 6.

4. Place the bottles containing buffer A and buffer B in the solvent reservoir behind the pump and UV monitor. Connect tubing from the buffers to the proportioning valve, as illustrated in Figure 5.

5. Use Figure 5 as a guide for plumbing the porportioning valve to mixer, mixer to pump, and diverter valve to fraction collector and waste.

6. Place the UV monitor optics module on the rack. Connect tubing from the UV outlet to the diverter valve and the UV inlet to the injector valve, at the cartridge position, as illustrated in Figure 6.

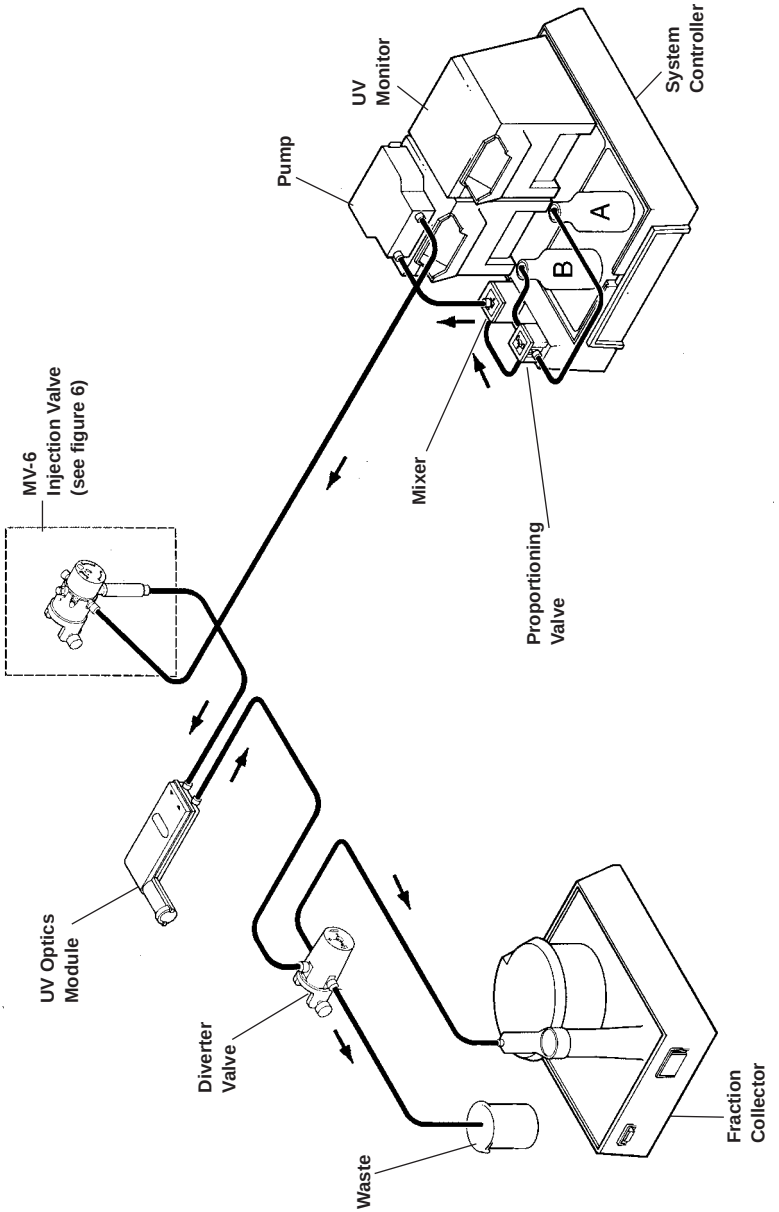


Fig. 5. The standard Econo System plumbing diagram. Minimize tubing lengths between components to reduce dead volume.

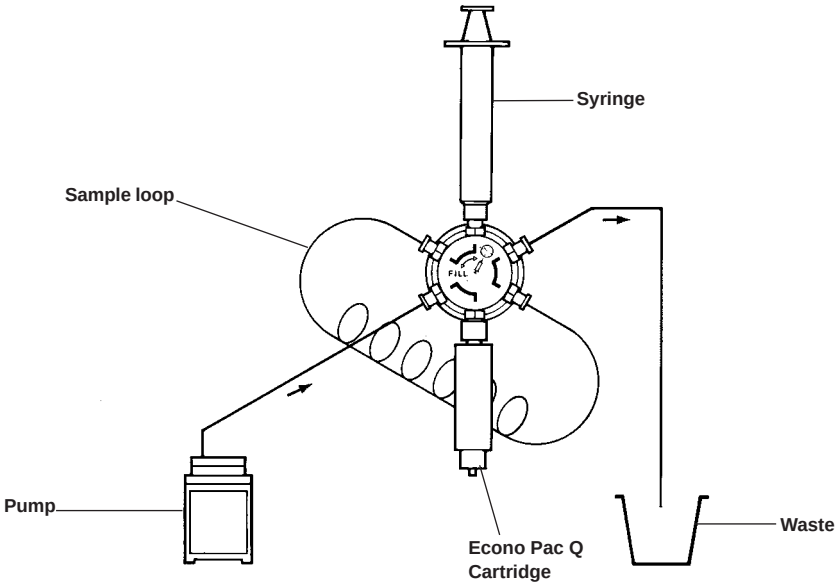


Fig. 6. MV-6 manual injection valve plumbing guide. The valve is in the "load" position (full counter-clockwise position). Do not connect the Econo-Pac Q cartridge to the valve at this time.

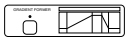
Pump Platen Adjustment

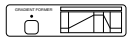
The pump platen is a metal screw located on the front left corner of the pump head (Figure 4). The pump platen should always be adjusted when new tubing has been installed in the pump head to insure a smooth and accurate flow rate.

1. Press the Tubing Selector key on the pump to select 1.6 mm tubing.
2. Set the flow rate on the pump at 1.5 ml/min by using the Arrow keys.
3. Turn the platen adjustment screw counter-clockwise until the stop is reached.
4. Turn the screw approximately three complete turns in the clockwise direction.
5. Start the pump by pressing the pump Run key.
6. This procedure should produce a smooth, even flow rate of buffer through the tubing. If the flow is not smooth then continue to adjust the platen adjustment screw clockwise until the flow appears to be even.

Purging the System

1. Press the Purge  key on the pump. The pump will run at maximum speed allowing air to be purged from the buffer lines of your system. Allow the system to run until air is purged from the entire system. (Note: if buffer A does not flow and bubbles appear from the tubing in the buffer A bottles, stop the pump and press the Direction  key to change the direction of the pump head.

2. With the system still running at purge speed, press the Gradient Former  key once to switch from buffer A to buffer B. The pump will display “b” to indicate the gradient proportioning valve has switched from buffer A to buffer B. A "click" will be heard as the valve switches.

3. With the pump still running, press the Gradient Former  key again to switch from buffer B back to buffer A. The disappearance of “b” on the pump display will indicate that the pump is now pumping buffer A.

4. Completely purge the MV-6 valve sample loop (the valve should already be in the counter-clockwise “load” position). Rotate the valve clockwise to the “inject” position to purge the inject port and sample loop.

5. When the sample loop has been purged of air, return the MV-6 valve to the counter-clockwise “load” position. Allow the system to run until all air is purged from the tubing lines. Press the Run  key to stop the pump.

Section 4
The Protein Separation

This Starter Kit allows you to separate a premixed anion exchange standard containing equine myoglobin, conalbumin, chicken ovalbumin, and soybean trypsin inhibitor, using a 1 ml Econo-Pac Q cartridge. Equine myoglobin is not retained on the Econo-Pac Q cartridge and elutes in the void volume. Conalbumin, chicken ovalbumin, and soybean trypsin inhibitor bind to the Q cartridge and require increased salt concentrations for elution. Conalbumin elutes at 10% buffer B, chickent ovalbumin elutes at 30% buffer B and soybean trypsin inhibitor requires greater than 30% buffer B for elution. The separation requires approximately 20 minutes.

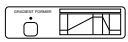
Run Conditions

Buffer A:	25 mM Tris-HCl, pH 8.1
Buffer B:	25 mM Tris-HCl, pH 8.1, 0.5 M NaCl
Flow rate:	1.5 ml/min
Sample volume:	1.5 ml
UV range:	0.1 AUFS
Chart recorder speed:	12 cm/hr

Section 5
Sample Preparation

1. Remove the aluminum cap from the Anion Exchange Standard vial. Slowly remove the rubber plug from the standard vial (the contents may be under vacuum).
2. Add 1.5 ml of prepared Buffer A to the vial.
3. Replace the rubber stopper and invert the vial several times to solubilize the protein standards.
4. Transfer the solubilized standards from the vial into a 15 ml screw top disposable sample tube. Add 8.5 ml of buffer A to the standard solution, cap the tube and invert several times to mix. Total volume is 10 ml.
5. The prepared protein sample can be stored at 4 °C for several days.

Section 6
Installing the Econo-Pac Q Cartridge

1. Set the pump flow rate to 1.5 ml/min using the Arrow keys and start the pump by pressing the Run  key.
2. Remove the blue cap from the inlet end of the Econo-Pac cartridge.
3. With the pump running, connect the inlet end of the Econo-Pac cartridge directly to the male luer fitting on the MV-6 injector valve (See Figure 6).
4. Immediately remove the end cap from the outlet end of the cartridge.
5. Plumb the outlet of the cartridge to the UV monitor optics module (figure 5).
6. Wash the cartridge with buffer A for 2 minutes.
7. Press the Gradient Former  key to change to buffer B(denoted by a “b” on the pump display.

8. Wash the cartridge with buffer B for 10 minutes.
9. Press the Gradient Former key once again to select buffer A. Re-equilibrate the cartridge with buffer A for 5 minutes and then stop the pump by pressing the Run key.

   You are now ready to program the Econo System.

Section 7
Programming a Linear Gradient

The Econo System software must be in "standard" mode to program the gradient separation presented in this section. To select the standard mode:

1. Press and hold down the Diverter Valve key, and immediately press the Gradient Former key, holding both for several seconds. Both keys are on the system controller. The pump display will flash either "Std" for standard mode or "Enh" for enhanced mode.
2. Use the Arrow keys located on the pump to select the "Std" mode. The display will flash "std".
3. Press the Run key to enter the standard mode. You are now ready to begin programming the method.

Setting Up the Method

The gradient method for the separation is:

0% buffer B at time 0

0% buffer B to 20% buffer B at 6 minutes

20% buffer B to 60% buffer B at 9 minutes

60% buffer B to 100% buffer B at 11 minutes

0% buffer B at 14 minutes

0% buffer B at 20 minutes

Figure 7 is a linear plot of the buffer B concentration vs time for this method. A plot of this type gives you a visual representation of the gradient over time.

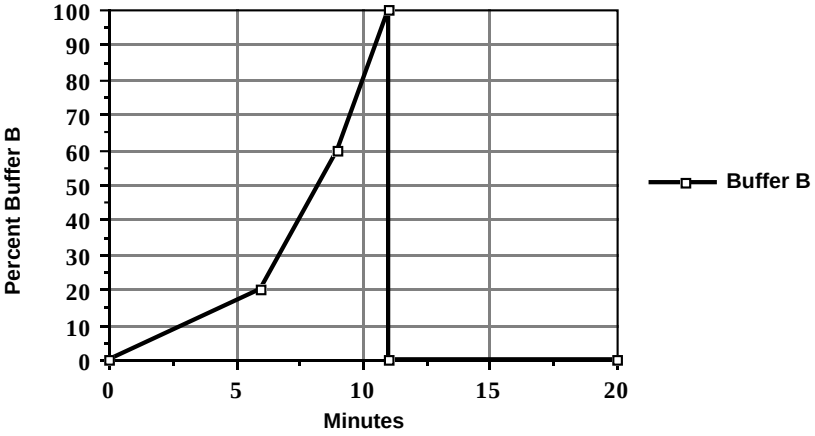


Fig. 7. Plot of buffer B concentration vs time for this method.

A programming table can also be used to help plan and organize any chromatographic separation program. A method on the standard Econo System has a maximum of five steps defined by six programmable inflection points (Table 1). Inflection points act as a transition point between each step of the program. Each inflection point requires that you enter a time value and the percentage of buffer B that you want at that time.

Table 1. Program table for the standard Econo System linear gradient separation.

Inflection Point	Cumulative Time (min)	%B
1	0	0%
2	6	20%
3	9	60%
4	11	100%
5	14	0%
6	20	0%

To program the method, first press the Gradient Former key located on the system controller. The minute indicator light on the pump and the pump display flashing "O" indicates that the pump is displaying the start time of “0” minutes. Follow the procedure outlined in Table 2 to enter the complete method program. Use the Arrow keys to enter time and buffer B values at each step.

Table 2. Step by step programming instructions for the standard Econo System linear gradient separation.

Inflection Point	Select (using arrow keys)	Pump Display	Key to Press
1	default "0" minutes	flashing "0" min	Gradient
1	0% buffer B	b 0%	Gradient
2	6 minutes	6 min	Gradient
2	20% buffer B	b 20%	Gradient
3	9 minutes	9 min	Gradient
3	60% buffer B	b 60%	Gradient
4	11 minutes	11 min	Gradient
4	100% buffer B	b 100%	Gradient
5	14 minutes	14 min	Gradient
5	0% buffer B	b 0%	Gradient
6	20 minutes	20 min	Gradient
6	0% buffer B	b 0%	Gradient

The gradient former indicator light will remain on, indicating the program is ready.

Section 8
Programming the Econo System to Collect Fractions

The fraction collector can be programmed to collect fractions for the entire run, using time windows or only when the UV absorbance is greater than a specified percent of full scale (threshold detection). For this method, the fraction size will be set at 1.0 ml fractions and the entire 20 minute method will be collected.

1. Press the Fraction Collector  key on the pump. Both the “ml” indicator light and “fraction size” indicator light will flash. Enter the fraction volume (1.0 ml) using the Arrow  keys located on the pump.
2. Press the Fraction Collector  key again. Enter a volume for V_o (collection delay volume) of 0.0 ml.
3. Press the Fraction Collector  key. The total run volume will be displayed (30 ml) (calculated by the method entered previously). This will be flashing and cannot be changed.

4. Press the Fraction Collector key again. The estimated maximum number of collection tubes required will be displayed (F 30). Note: if the total run volume or number of fractions is not 30, then either the flow rate or the length of the programmed method has not been entered correctly.

5. Press the Fraction Collector key. The fraction collector indicator light will remain on indicating the Econo System is ready to collect fractions. The pump display will show the set flow rate (in ml/min).

6. Place fraction collection tubes in each of the first 30 spaces in the fraction collector carousel. Position tube #1 under the fraction collector drop former.

Section 9
System Check List
Prior to Running the Method

Prior to running the method, the following system parameters should be checked.

Electrical

1) Are all the electrical connections correct (see Figure 3)?

Plumbing

1) Is the system plumbed correctly (see Figure 5)?
2) Is the MV-6 injector valve plumbed correctly (see Figure 6)?
3) Are all tubing lines purged of air?

Pump

1) Is the pump running in the correct direction?
2) Does the tubing Calibration, located on the front of the pump, match the tubing ID installed in the pump head?
3) Is the Fraction Collector key indicator light on?
4) With the pump running, is the Program Run key flashing?
5) Is the method entered correctly?
6) Is the pump flow rate set at 1.5 ml/min?
7) Has the pump platen been adjusted (See Section 3)?

UV Monitor

1) Set the UV monitor range setting to 0.1 AUFS using the Arrow keys located on the UV monitor

2) Zero the UV monitor against buffer A by pressing and holding the Auto-Zero key until the light flashes. Perform this step while buffer is pumping.

System Controller

1) Is the Gradient Former key indicator light on? If the light is on, the programmed method is activated.

Chart Recorder

1) Set all the control switches on the chart recorder to the green labeled values.

2) Set the chart paper speed to 12 cm/hr.

3) If the UV monitor has been zeroed, position the pen at the 10% line. Turn the baseline adjust dial until the pen is positioned.

Fraction Collector

1) Are there at least 30 tubes in the rack?

2 Is the first tube in position under the drop former?

3) Does the fraction collector display three dashes (---), indicating that it is properly connected to the Econo System?

Section 10
Injecting Sample and Starting the Method

After checking all system parameters (Section 10) the sample can be injected and the method started.

1. With the Gradient Former indicator light on, press the Run key to start the pump. The set flow rate will be displayed on the pump (1.5 ml/min). The chart recorder pen(s) will automatically drop and begin recording.

2. Position the MV-6 injection valve in the “load” position (counter-clockwise, Figure 6).

3. Load the sample loop with approximately 1.5 ml of the prepared protein sample using the syringe provided with the kit. This volume should be enough to fill the sample loop. Do not remove the syringe from the MV-6 injection valve after sample has been loaded, otherwise, the sample will drain from the loop into the waste.

4. To inject the sample onto the column, turn the MV-6 injection valve to the "inject" position (clockwise). Immediately press the flashing Program Run key to start the programmed method. An event mark will appear on the recorder when the Program Run key is pressed.

The system is now running the programmed method. After several minutes you will hear the proportioning valve clicking as it mixes the programmed proportion of buffer A and buffer B. When the program is complete the system will produce an audible “beep”. The pump and chart recorder will automatically stop, and the pump display will read “end”.

Part II Automated Econo System

The following instructions assume that you have a complete Automated Econo System, including:

- **Econo Buffer Selector**
- **Econo Gradient Monitor**
- **Econo Pump**
- **Econo UV Monitor**
- **Econo System Controller**
- **Model 2110 Fraction Collector**
- **Dual-Pen Econo Recorder**
- **Econo System Rack**
- **Econo System Organizer**

Section 1
Buffer Preparation

If you've completed Part I of the starter kit, the buffers and protein standard are already prepared except for the 0.1 N NaOH. If you did not complete Part I, refer to Part I, Section 1 for instructions on buffer and sample preparation.

Section 2
Setting Up The System

Setting up the Automated Econo System involves the addition of the gradient monitor and buffer selector to the Standard Econo System. If you've already completed Part I of this starter kit, you are now ready to set up the Automated Econo System. If you did not complete Part I of the starter kit, see Part I, Section 2 to set up the standard Econo System components.

1. Position the buffer selector and gradient monitor underneath the system organizer. Metal tabs on the underside of the system organizer will prevent these components from sliding to the rear (Figure 8).
2. Position the chart recorder, fraction collector and rack as illustrated in Figure 8. The solvent reservior included with the system controller sits on top of the system controller (behind the pump and UV monitor).
3. Connect the gradient monitor and buffer selector to the system controller as illustrated in Figure 9. Use the power adaptors to power up the gradient monitor and buffer selector.

Fig. 8. Positioning of the Automated Econo System.

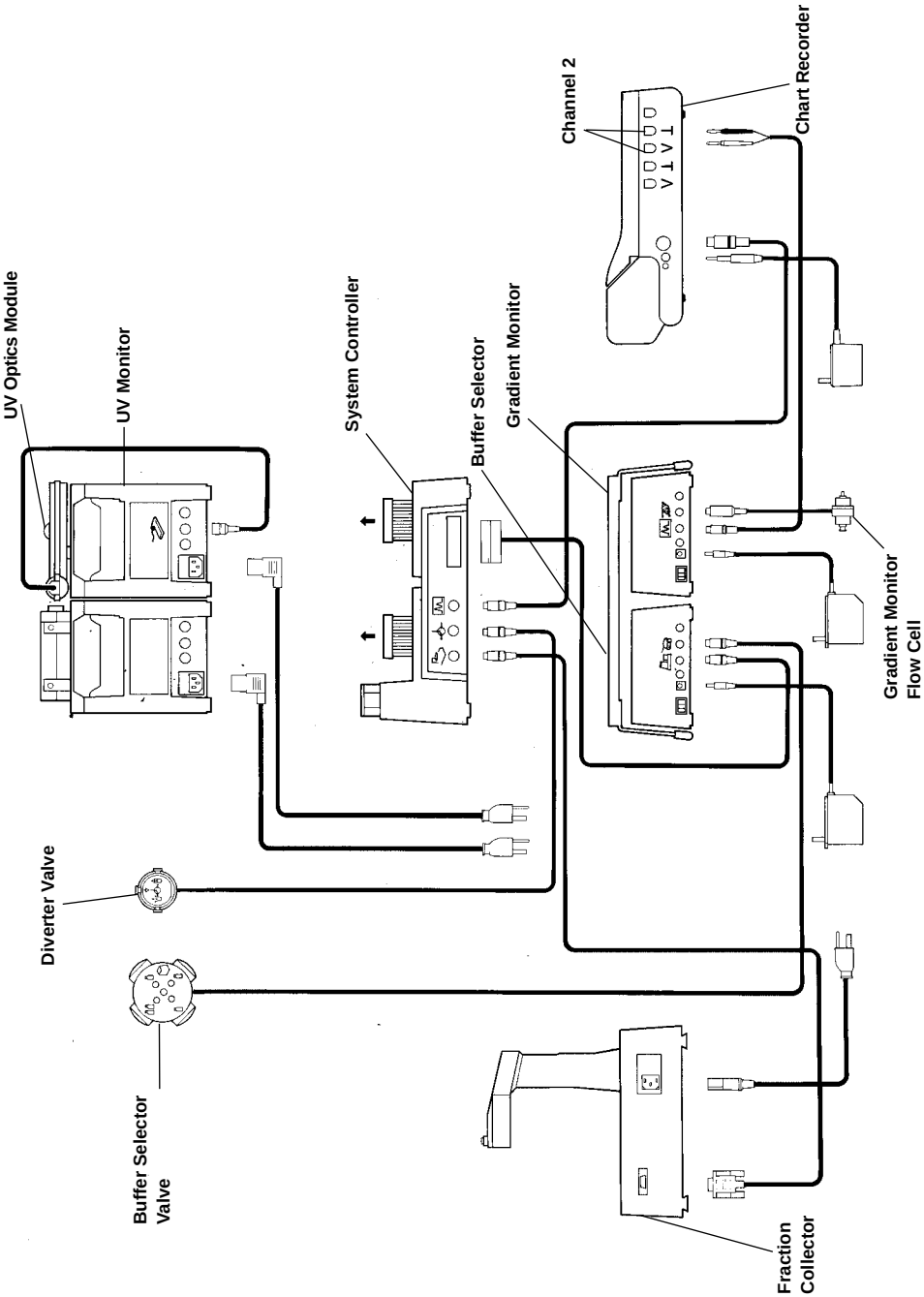


Fig. 9. Automated Econo System electrical connections.

Powering Up

Turn the power on for all components. Power switches are on the bottom panels of the pump and UV monitor, and on the rear panels of both the buffer selector and gradient monitor.

Section 3
System Plumbing, Purging and Platen Adjustment

System Plumbing

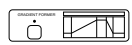
Plumb the Automated Econo System as described in Figure 10. Check to insure that 1.6 mm ID tubing is installed in the pump head. Check to ensure that all luer fittings are tight and that the total length of tubing used between each component is minimized to reduce dead volumes. The MV-6 valve is recommended for injection of sample volumes less than 5 ml and the buffer selector valve is recommended for injection of sample volumes greater than 5 ml. For this method, only 1.5 ml of the sample will be loaded using the buffer selector to demonstrate how to use the automatic valve.

Purging the System

1. Connect all the buffer lines prior to purging the Automated Econo System. The protein sample will be plumbed as buffer "C". When purging, be careful not to waste the protein sample and be sure to disconnect the Econo-Pac cartridge before purging.

2. Press the Purge  key located on the pump. The pump will run at maximum speed allowing air to be purged from the buffer lines of your system. Allow the system to run until air is purged from the entire system. (Note: if buffer A does not flow and bubbles appear from the tubing in the buffer A bottles, stop the pump and press the Direction  key to change the direction of the pump head).

3. With the system still running at purge speed, press the Gradient Former  key once to switch from the buffer A line to the buffer B line. The buffer selector will display “b” to indicate the gradient proportioning valve has switched from buffer A to buffer B.

4. With the pump still running, press the Gradient Former  key again to switch from buffer B to buffer C. The buffer selector will display “c” to indicate that buffer C is flowing through the pump. Don't waste sample when purging buffer C. Continue this procedure to completely purge the buffer lines for buffer D and E.

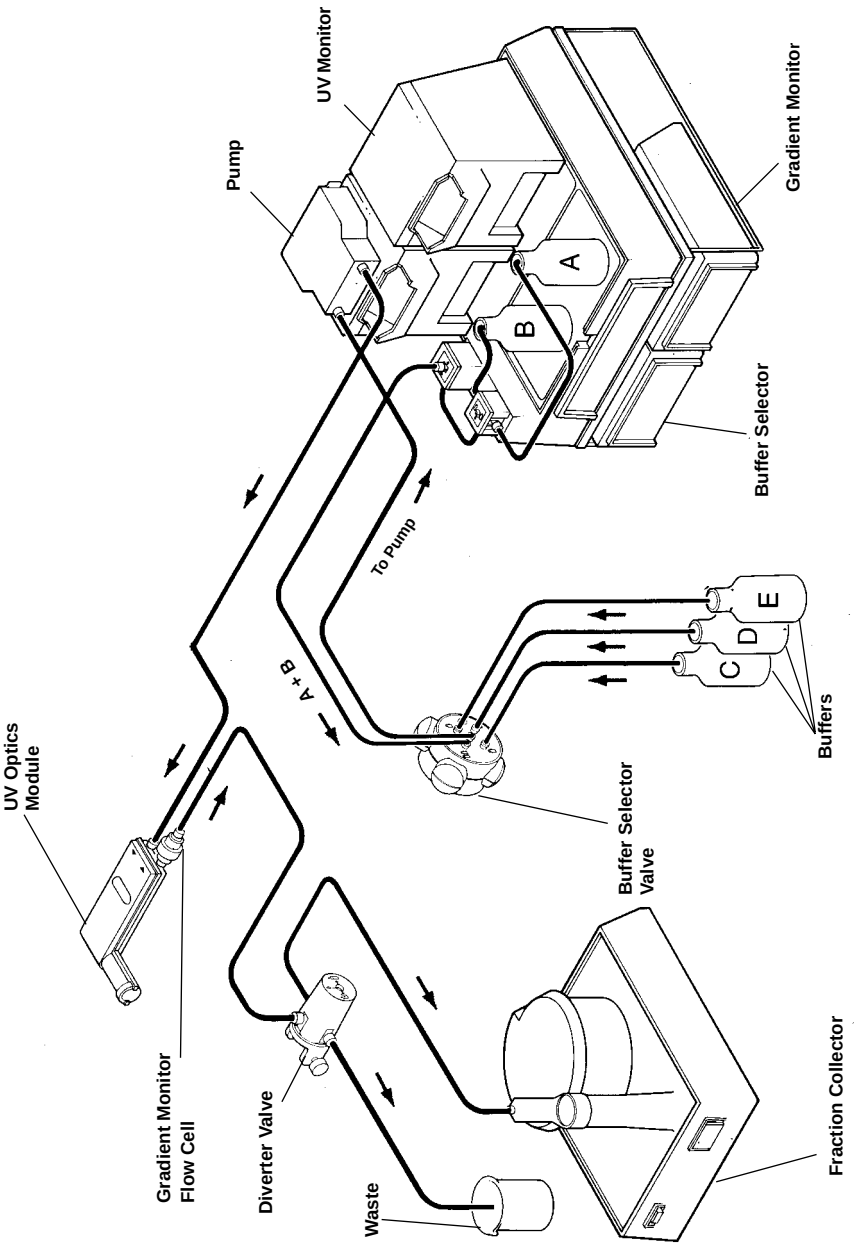


Fig. 10. Automated Econo System plumbing diagram with buffer selector connections. For this separation the cartridge will be placed in line between the pump and the UV optics module.

5. When all air is removed from the system, press the Run  key to stop the pump.

Section 4
The Protein Separation

This Starter Kit allows you to separate a premixed anion exchange standard containing equine myoglobin, conalbumin, chicken ovalbumin, and soybean trypsin inhibitor, using the Econo-Pac Q cartridge. Equine myoglobin is not retained on the Econo-Pac Q cartridge and elutes in the void volume. Conalbumin, chicken ovalbumin, and soybean trypsin inhibitor bind to the Q cartridge and require increased salt concentrations for elution. Conalbumin elutes at 10% buffer B, chickent ovalbumin elutes at 30% buffer B and soybean trypsin inhibitor requires greater than 30% buffer B for elution. Part II of the starter kit allows you to program a 30 minute linear gradient method with the Automated Econo System.

Buffers

Buffer A = 25 mM Tris-HCl, pH 8.1

Buffer B = 25 mM Tris-HCl, pH 8.1, 0.5M NaCl

Buffer C = Protein Sample

Buffer D = 0.1N NaOH

Buffer E = water

Gradient Running Conditions

Flow rate:	1.5 ml/min
Sample volume:	1.5 ml
UV range:	0.05 AUFS
Chart recorder speed:	12 cm/hr

Section 5
Installation and Equilibration of the Econo-Pac Q Cartridge

With the Automated Econo System, the Econo-Pac Q cartridge can be positioned anywhere between the pump and the inlet side of the UV monitor optics module (Figure 10). The cartridge should be run in a vertical position. To plumb

the cartridge, simply connect tubing from the pump to the top of the cartridge and from the bottom of the cartridge to the inlet of the UV optics module. It is important to minimize the length of tubing used between the pump, cartridge and the UV monitor to minimize the dead volume.

Section 6
Programming a Linear Gradient

The Econo System software must be in “Enh” enhanced mode to program control of the buffer selector valve pod. To set the enhanced mode:

1. Press and hold down the Diverter Valve key and immediately press the Gradient Former key. Hold both keys for several seconds until the pump display begins flashing. The pump display will flash either “Std” for standard mode or “Enh” for enhanced mode.
2. Use the Arrow keys to select enhanced mode. The pump display will flash “Enh”.
3. Press the Run key to enter the enhanced mode. You are now ready to begin programming the method.

Setting Up the Method

The gradient method for the separation is:

100% Buffer A at time 0

100% buffer C at 1 minute

100% buffer A at 2 minutes

0% buffer B at 4 minutes

40% buffer B at 20 minutes

100% buffer B at 20 minutes

100% buffer B at 23 minutes

100% buffer D at 23 minutes

100% buffer A at 25 minutes

100% buffer A at 30 minutes

The Automated Econo System Method Table (Table 3) is used to help plan and organize your chromatographic separation program

Table 3. Program table for the Automated Econo System linear gradient separation with separate gradient programming block (L).

Inflection Point	Cumulative Time (min)	Valve/ Buffer
1	0	A
2	1	C
3	2	A
4	4	L
5	23	D
6	25	A
7	30	A
8	30	A
9	30	End

Inflection Point	Cumulative Time (min)	%B
1 (L)	4	0%
2 (L)	20	40%
3 (L)	20	100%
4 (L)	23	100%
5 (L)	23	100%
6 (L)	23	100%

The Automated Econo System has a total of nine inflection points which define eight unique programmable steps. Inflection points act as the transition point between each step of the program. Each inflection point is required to define both the start time and the buffer for that step.

The Automated Econo System has the ability to integrate the standard Econo System binary gradient programming block (L, same as programmed in Part I of this manual). This feature gives the Automated Econo System the ability to not only control five different buffers but also to execute a 5 step binary gradient of buffer A and B at any step of the program.

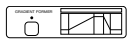
To program the method, first press the Gradient Former  key located on the system controller. The minute indicator light on the pump and the pump display flashing "O" indicates that the pump is displaying the start time of “0” minutes. The flashing “1” on the buffer selector indicates that you are at inflection point 1 of the method in Table 3 or 4. The shaded region of Table 4 represents the gradient block (L) of the method. Follow the procedure outlined in Table 4 to enter the complete method program. Use the Arrow  keys located on the pump to enter time, buffers and percent buffer B at each step.

Table 4. Parameters for the automated linear gradient program

Inflection Point (buffer selector display)	Select (using arrow keys)	Key to Press	Select (using arrow keys)	Key to Press
1	Flashing time "0"	Gradient	A	Gradient
2	1 minute	Gradient	C	Gradient
3	2 minutes	Gradient	A	Gradient
4	4 minutes	Gradient	L	Gradient
4 (L1)	4 minutes	Gradient	0% B	Gradient
4 (L2)	20 minutes	Gradient	40% B	Gradient
4 (L3)	20 minutes	Gradient	100% B	Gradient
4 (L4)	23 minutes	Gradient	100% B	Gradient
4 (L5)	23 minutes	Gradient	100% B	Gradient
4 (L6)	23 minutes	Gradient	100% B	Gradient
5	23 minutes	Gradient	D	Gradient
6	25 minutes	Gradient	A	Gradient
7	30 minutes	Gradient	A	Gradient
8	30 minutes	Gradient	A	Gradient
9	30 minutes	Gradient	End	

Section 7
Gradient Monitor Calibration

Set the gradient monitor to gradient mode by pressing the Mode Selector key until the gradient mode indicator light is on.

1. Begin calibration by pressing the Gradient Calibration CAL key located on the gradient monitor. The “buffer A” indicator light on the gradient monitor will flash.
2. Disconnect the gradient monitor flow cell from the Econo System plumbing, and, using a 10 ml syringe, pass buffer A through the flow cell until the display reading stabilizes.
3. Press the Calibration CAL key to enter the value for buffer A value. The “buffer B” indicator light will now flash. Using a syringe, pass some of buffer B through the flow cell until the display reading stabilizes.
4. Press the Calibration CAL key to enter the buffer B value. The gradient monitor is now calibrated for buffer A and B.
5. Connect the gradient monitor flow cell to the outlet of the UV optics module.

Section 8
System Check Prior to Running the Method

Prior to running the method, the following system parameters should be verified.

Electrical

1) Are all the electrical connections correct (see Figure 9)?

Plumbing

1) Is the system plumbed correctly (see Figure 10)?

Pump

1) Is the pump running in the correct direction?
2) Does the Tubing Calibration, located on the front of the pump, match the ID of the tubing installed in the pump head?
3) Is the Fraction Collector key indicator light on?
4) With the pump running, is the Program Run key flashing?
5) Is the method entered correctly?
6) Is the pump flow rate set at 1.5 ml/min?
7) Is the pump platen adjusted (See Part I, Section 3)?

UV Monitor

1) Set the UV monitor range setting to 0.05 AUFS using the Arrow keys located on the UV monitor.
2) Zero the UV monitor against buffer A by pressing and holding the Auto-Zero key for 3 seconds until the light flashes, while buffer is pumping.

Buffer Selector

1) Is buffer selector valve pod correctly plumbed for buffers A+B, C, D, and E?

Gradient Monitor

1) Is the gradient monitor operating in gradient mode?
2) Has the gradient monitor been calibrated with buffer A and B?

System Controller

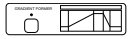
1) Is the Gradient Former  key indicator light on? If the light is on, the programmed method is activated.

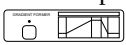
Chart Recorder

1) Set all the control switches on the chart recorder to the green labeled values.
2) Set the chart paper speed to 12 cm/hr.
3) If the UV monitor has been zeroed, position the pen at the 10% line. Turn the baseline adjust dial until the pen is positioned.

Fraction Collector

1) Are there at least 45 tubes in the rack?
2) Is the first tube in position under the drop former?
3) Does the fraction collector display three dashes (---), indicating that it is properly connected to the Econo System?

Section 9
Sample Injection/Method Start

1. Start the pump and set the flow rate to 1.5 ml/min. The Gradient Former  key indicator light on the controller must be on and the Program Run  key indicator light of the pump must be flashing to run a program.
2. Press the Program Run  key and the display will show an “n 1” which denotes the number of times the program will repeat. Press the Program Run  key again to confirm n=1 (the method will run only one time) and to start the method. An event mark will appear on the recorder.

Appendix A
Product Information

Accessories and Replacement Parts

Catalog Number	Product Description
Valves	
731-8237	**Model MV-6 6-Port Injection Valve**
731-8235	**Model SV-3 Diverter Valve**
Tubing	
731-8210	**Silicone Tubing,** 0.8 mm ID, 0.8 mm wall, 10 m
731-8211	**Silicone Tubing,** 1.6 mm ID, 0.8 mm wall, 10 m
731-8212	**Silicone Tubing,** 3.2 mm ID, 0.8 mm wall, 10 m
731-8214	**Tygon® Tubing,** 0.8 mm ID, 0.8 mm wall, 10 m
731-8215	**Tygon Tubing,** 1.6 mm ID, 0.8 mm wall, 10 m
731-8207	**PharMed® Tubing,** 0.8 mm ID, 1.0 mm wall, 10 m
731-8208	**PharMed Tubing,** 1.6 mm ID, 1.0 mm wall, 10 m
731-8209	**PharMed Tubing,** 3.2 mm ID, 1.0 mm wall, 10 m
Tubing Kits	
731-8240	**Silicone Tubing,** 0.8 mm, 20 pre-cut lengths; 4 red lock-rings, 4 female luer-fittings, 4 male luer-fittings
731-8241	**Silicone Tubing,** 1.6 mm, 20 pre-cut lengths; 4 orange lock-rings, 4 female luer-fittings, 4 male luer-fittings
731-8242	**Silicone Tubing,** 3.2 mm, 20 pre-cut lengths; 4 yellow lock-rings, 4 female luer-fittings, 4 male luer-fittings
731-8247	**PharMed Tubing,** 0.8 mm, 20 pre-cut lengths; 4 red lock-rings, 4 female luer-fittings, 4 male luer-fittings
731-8248	**PharMed Tubing,** 1.6 mm, 20 pre-cut lengths; 4 orange lock-rings, 4 female luer-fittings, 4 male luer-fittings
731-8249	**PharMed Tubing,** 3.2 mm, 20 pre-cut lengths; 4 yellow lock-rings, 4 female luer-fittings, 4 male luer-fittings

Catalog Number	Product Description
Fittings	
732-8220	**Low Pressure Fittings Kit,** includes over 250 male and female luer connectors, 2- and 3-way stopcocks, and tubing connectors
731-8221	**Female Luer with Barb for 0.8 mm ID Tubing,** 25
731-8222	**Female Luer with Barb for 1.6 mm ID Tubing,** 25
731-8223	**Female Luer with Barb for 3.2 mm ID Tubing,** 25
731-8224	**Male Luer with Barb for 0.8 mm ID Tubing,** 25
731-8225	**Male Luer with Barb for 1.6 mm ID Tubing,** 25
731-8226	**Male Luer with Barb for 3.2 mm ID Tubing,** 25
731-8228	**Female-to-Female Luer,** 10
731-8229	**Female-to-Female-to-Female Luer,** 10
731-8230	**Male-to-Male Luer,** 10
731-8232	**Female Luer Plugs,** 25
731-8233	**Male Luer Plugs,** 25
732-8102	**2-way Polycarbonate Stopcock,** 10
732-8103	**3-way Polycarbonate Stopcock,** 10
732-8107	**3-way Nylon Stopcock,** 10
732-8300	**Straight-through Connector,** 25
732-8302	**T-connector,** 25

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U.S. *(800) 4BIORAD* ***California*** *(510) 741-1000* ***Australia*** *02-805-5000* ***Austria*** *(1) 877 89 01* ***Belgium*** *09-385 55 11* ***Canada*** *(905) 712-2771* ***China*** *(01)2046622* ***Denmark*** *39 17 9947* ***Finland*** *90 804 2200* ***France*** *(1) 49 60 68 34* ***Germany*** *089 31884-0* ***India*** *91-11-461-0103* ***Italy*** *02-21609 1* ***Japan*** *03-5811-6270* ***Hong Kong*** *7893300* ***The Netherlands*** *0318-540666* ***New Zealand*** *09-443 3099* ***Singapore*** *(65) 272-9877* ***Spain*** *(91) 661 70 85* ***Sweden*** *46 (0) 735 83 00* ***Switzerland*** *01-809 55 55* ***United Kingdom*** *0800 181134*

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