

SOP: BioLogic LP Chromatography System Operation

Approvals:

Preparer: Lara Dowland
Reviewer: Cianna Cooper

Date: 10JUN18

Date: 12JUN18

1. Purpose:

1.1. Operation of the BioLogic LP Chromatography System.

2. Scope:

2.1. Applies to the BioLogic LP Chromatography System for purifying proteins.

3. Responsibilities:

3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

4.1. BioLogic LP Chromatography System Instruction Manual

5. Definitions:

6. Precautions: N/A

7. Materials:

7.1. >250ml Deionized Water

7.2. ~500ml Buffer A (Refer to the appropriate chromatography SOP)

7.3. ~500ml Buffer B (Refer to the appropriate chromatography SOP)

7.4. ~500ml Cleaning Solution (Refer to the appropriate chromatography SOP)

7.5. Container for Waste Fluid

7.6. Collection Tubes for Fraction Collector or collection containers

7.7. Column

7.8. Resin (Refer to the appropriate chromatography SOP)

8. Procedure:

8.1. Turn on BioLogic LP system (switch is in the front, on the lower left side of the system).

8.2. Turn on computer

8.3. Click on the LP DataView icon

8.4. Verify that the computer is communicating with the system as indicated by a green "Receive" circle on the upper right side of the computer screen.

8.5. Programming the Biologic LP Chromatography instrument

8.5.1. To enter program mode, press the **Prog** key. It is usually easier to modify an existing program than to program a new one. To modify an existing program, go to Step 8.5.9.

8.5.2. Select the **New Method** soft key.

8.5.3. Select **Time** or **Volume mode**. These are the units used to define actions within a method; once the selection is made it cannot be changed.

8.5.4. Press the **Add** soft key. Enter the desired parameters for duration, buffer and flow rate. Note – to program the Fraction Collector or Alarm without returning to Method list, press the **Collector** and **Alarm** soft keys respectively.

SOP: BioLogic LP Chromatography System Operation

8.5.4.1. Select Buffer – buffers are indicated by the letters available. The brackets around one the buffers [], indicates which buffer is currently selected. Use the **Prev** and **Next** keys to change the selection. **Grad** key is used to program a linear gradient in this step. **Mix** is used to program a mixture in this step. **Cancel** cancels the addition or modification of this step and returns to the Pump Table as it was originally. **OK** confirms the selected buffer as indicated by the brackets surrounding the letter and continues to the next step. The letters G and M stand for gradient and mixture. Gradient represents a linear gradient made from Buffers A and B and Mixture refers to a single solution made by mixing Buffers A and B.

8.5.4.2. Length of Step- the duration of the step is entered using the number keys. The duration is in units of time (minutes) or volume (ml).

8.5.4.3. Flow Rate – flow rate of this step is entered using the number soft keys. This is the final entry for a pump step.

8.5.4.4. Continue entering buffer steps.

8.5.5. Press the **Collector** instrument key while in Program mode. Select Collection mode from the following choices:

All – collect all mode collect fractions throughout the method.

Threshold – collect peaks by defining a threshold value in absorbance units (AU).

Windows – allows you to specify periods called “windows” within the method when fractions will be collected.

Thresh+Win – selects threshold plus collection windows.

8.5.5.1. Fraction Collection Summary – shows the current collection mode and an estimate of the number of fractions which will be collected. **Edit** key allows the fraction collection parameters to be viewed and changed. **Erase** key removes the existing fraction collection programming from the method. **New Mode** allows the collection mode to be changed by displaying the Select Collection Mode screen. **OK** returns to the Method List.

8.5.6. Press the **Alarm** key to set alarms during the program. Up to three alarms can be programmed in a method. Units will be either time or volume, depending on the choice made when the method was created.

8.5.6.1. The selected alarm is shown with an arrow (>) pointing to the alarm.

Use the **Prev** and **Next** keys to move the selection up and down the list.

Edit allows the parameters of the selected alarm to y changed. **Delete** removes the selected alarm from the table (* the alarm cannot be retrieved).

Add allows a new alarm to be added at the end of the table. The next time the Alarm Table is viewed, the alarms will be rearranged

in the order of occurrence. **OK** indicates the table is complete and returns to the Methods List.

SOP: BioLogic LP Chromatography System Operation

8.5.6.2. Alarm Settings – enter the time or volume for the alarm to occur using the number keys.

8.5.6.2.1. Hold On – programs the method to hold when the alarm occurs.

8.5.6.2.2. Hold Off – programs the method to run normally with the alarm occurs.

8.5.6.2.3. Cancel – returns to the alarm table without adding or modifying the alarm.

8.5.6.2.4. OK – returns to the Alarm Table with the new or modified alarm in the table.

8.5.7. Entering a Name for the New Method – method names can consist of up to 12 character and can include upper case letters, number and spaces.

8.5.7.1. Method Name Entry screen – allows method names to be entered. The underscore shown within the method name on the top line is the insertion point where the next character will be placed. Use the **Prev** and **Next** keys to move the insertion point. To enter a letter character, select the soft key which shows the range containing the desired letter followed by the number key indicated after selecting the range. To use a number, press the number key directly. Use the decimal (.) key to place a space in the name. Press the **Done** key to return to the List of Methods after the name has been entered.

8.5.8. Viewing and Editing a Method – press the **View Method** key; the Method List will be displayed. Each step of the method can be viewed and edited to make whatever changes are necessary. When the contents of the method are correct, press the **Done** key. If changes were made, there will be a prompt to save the method. Refer to step 8.5.8 to save the method with a new name.

8.5.8.1. The numbered steps indicate the pump step within the method. Below each step are any fraction collection events or alarm events which will occur during the step. Use the **Prev** and **Next** keys to scroll through the list of steps. Press the **Edit** softkey to make changes.

8.6. Pump Calibration

8.6.1. Based on the desired flow rate, select the appropriate tubing for the pump.

Flow rates of 0.04-0.8 ml/min require 0.8mm tubing

Flow rates of 0.2-4.0 ml/min require 1.6mm tubing

Flow rates of 0.8-15.0 ml/min require 3.2mm tubing.

8.6.2. Verify that the correct tubing is in the pump

8.6.2.1. Remove the platen by lifting the grey handle (figure 2).

8.6.2.2. If necessary, insert the correct tubing.

8.6.2.3. Replace platen and lock into place.

8.6.2.4. If tubing was replaced readjust the platen and recalibrate the pump

8.6.2.4.1. Loosen the platen adjust screw located on the top of the pump (fig. 2) by turning counterclockwise until there is slight resistance.

SOP: BioLogic LP Chromatography System Operation

- 8.6.2.4.2. Tighten the platen screw clockwise the appropriate number of COMPLETE turns.
 - 0.8mm tubing requires 5 turns
 - 1.6mm tubing requires 4 turns
 - 3.2mm tubing requires 3 turns
- 8.6.2.4.3. Recalibrate the pump
 - 8.6.2.4.3.1. Press the MANUAL mode key
 - 8.6.2.4.3.2. Press the PUMP instrument key
 - 8.6.2.4.3.3. Select FLOW, then select CALIBRATE
 - 8.6.2.4.3.4. Select the appropriate tubing size.
 - 8.6.2.4.3.5. Select NOMINAL.
- 8.7. **Purge the System** – this is done without the column attached.
 - 8.7.1. Place each buffer line into a container filled with deionized water or buffer.
 - 8.7.2. Attach the column inlet tube directly to the column outlet tube.
 - 8.7.3. Press the MANUAL mode key.
 - 8.7.4. Select BUFFER, starting with A.
 - 8.7.5. Select OK.
 - 8.7.6. Select PURGE.
 - 8.7.7. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
 - 8.7.8. Select BUFFER.
 - 8.7.9. Using the arrow key, select B.
 - 8.7.10. Select OK.
 - 8.7.11. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
 - 8.7.12. Select Buffer. Select C.
 - 8.7.13. Select OK.
 - 8.7.14. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
 - 8.7.14.1. While the system is running, zero the UV Monitor.
 - 8.7.14.2. Press the UV instrument key.
 - 8.7.14.3. Select ZERO.
 - 8.7.14.4. Verify that the absorbance changes to zero on the display panel of the controller.
 - 8.7.14.5. Press the PUMP instrument key.
 - 8.7.14.6. Select STOP.
- 8.8. **Attach the Column/Equilibration of the Column**
 - 8.8.1. Disconnect the column inlet and outlet tubing from the tubing connector.
 - 8.8.2. Attach the column inlet tubing from the injector valve to the top of the column.
 - 8.8.3. Attach the column outlet tubing to the bottom of the column.
 - 8.8.4. Place tubing from the 3-way valve side port to the waste container.
 - 8.8.5. Press MANUAL mode key

SOP: BioLogic LP Chromatography System Operation

- 8.8.6. Select PURGE
- 8.8.7. Select START
- 8.8.8. Allow buffer to drip into the waste container from the side port until air bubbles are completely absent in the inlet tubing.
- 8.8.9. Select STOP.
- 8.9. Run the Column**
 - 8.9.1. Place all lines in the appropriate buffers/solutions as per the appropriate chromatography SOP.
 - 8.9.2. Press the PROGRAM mode key
 - 8.9.3. Select LIST METHODS
 - 8.9.4. Using the arrow keys, select the correct method as per the appropriate chromatography SOP.
 - 8.9.5. Select OPEN
 - 8.9.6. Using the arrow keys, verify that the method has not been changed.
 - 8.9.7. Select DONE
 - 8.9.8. Press the “Run” mode key
 - 8.9.9. System will have a 10 second delay.
 - 8.9.10. Verify that the computer is recording data by the appearance of an S symbol on the graph.
 - 8.9.10.1. If the S is not present, click the “record” button on the toolbar on the computer.
- 8.10. Clean the Column**
 - 8.10.1. Place buffer lines into the appropriate cleaning solution (refer to the Isolation of anti IL-8 mAb by Protein A Affinity Chromatography SOP).
 - 8.10.2. Run the appropriate cleaning method (refer to Isolation of anti IL-8 mAb by Protein A Affinity Chromatography SOP).
- 8.11. Clean and Store the System**
 - 8.11.1. If the system will be used again with the same column within a few days, it may be stored as is after a run.
 - 8.11.1.1. Turn off the system and turn off the computer
 - 8.11.2. If the system will not be used within a few days it must be flushed with water then 20% ethanol.
 - 8.11.2.1. Disconnect the column
 - 8.11.2.2. Attach the column inlet tube directly to the column outlet tube.
 - 8.11.2.3. Place each buffer line into a container filled with deionized water.
 - 8.11.2.4. Press the MANUAL mode key
 - 8.11.2.5. Select BUFFER.
 - 8.11.2.6. Select MIX
 - 8.11.2.7. Type in 50% B
 - 8.11.2.8. Select OK
 - 8.11.2.9. Select PURGE.
 - 8.11.2.10. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).

SOP: BioLogic LP Chromatography System Operation

- 8.11.2.11. Select BUFFER
- 8.11.2.12. Using the arrow key, select C
- 8.11.2.13. Select OK
- 8.11.2.14. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
- 8.11.2.15. Select STOP
- 8.11.2.16. Repeat steps 8.11.2.3 through 8.11.2.15 using 20%ethanol.

9. Attachments:

- 9.1. Figure 1: Controller Front Panel
- 9.2. Figure 2: Controller Pump
- 9.3. Figure 3: Photograph of the system with parts labeled

10. History:

Name	Date	Amendment
Lara Dowland	12Jun18	Initial release

SOP: BioLogic LP Chromatography System Operation

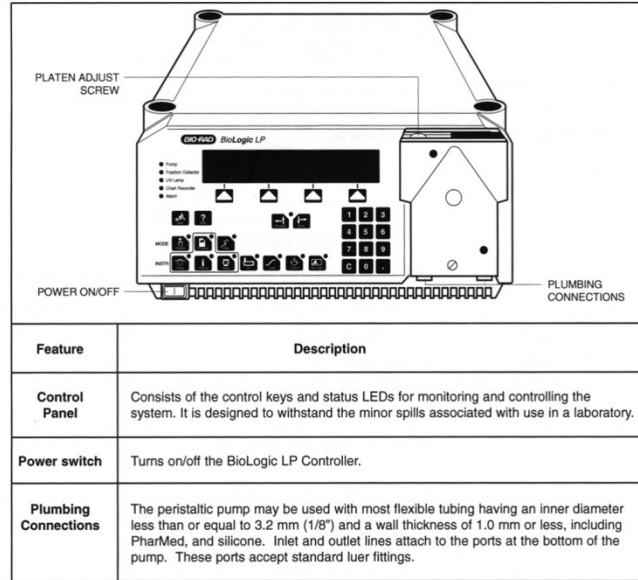


Figure 1: Controller Front Panel

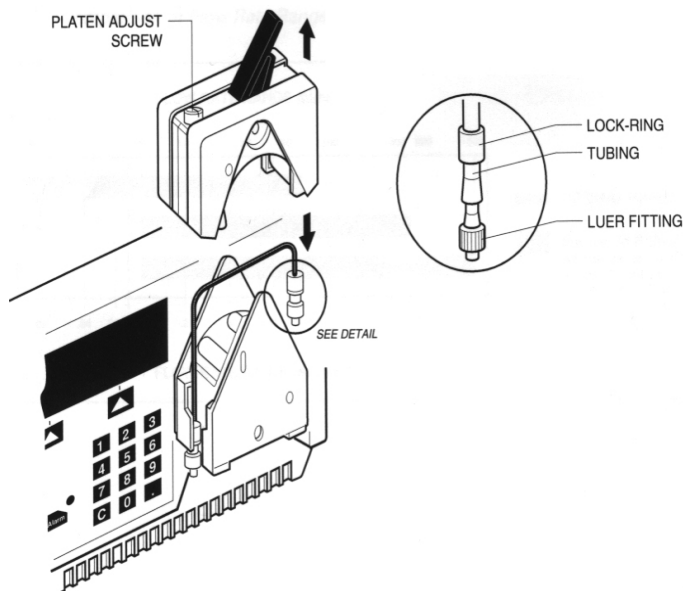


Figure 2: Controller Pump

SOP: BioLogic LP Chromatography System Operation

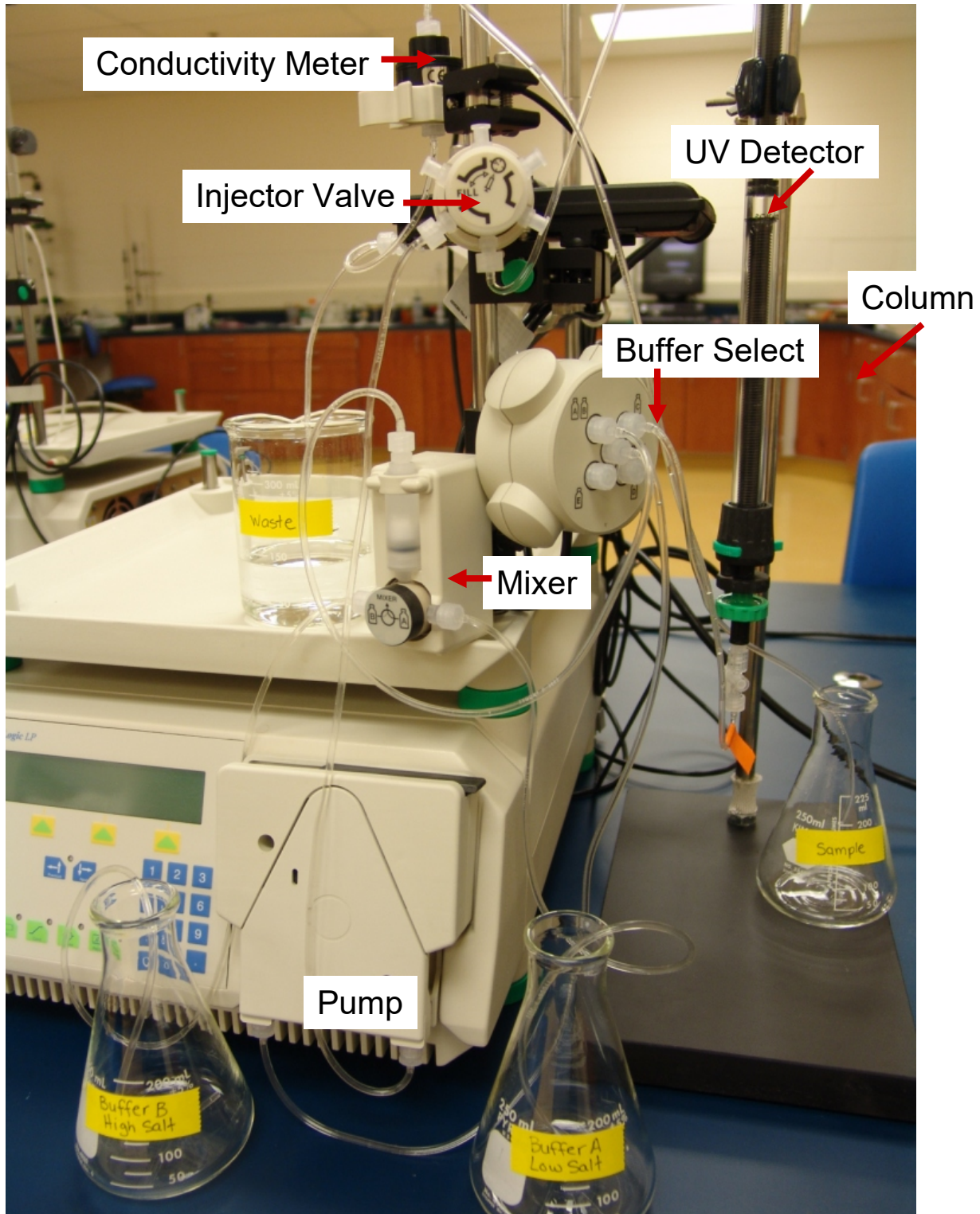


Figure 3: Photograph of the system with parts labeled