

SOP: BioLogic LP HIC Chromatography of Green Fluorescent Protein (GFP)

Approvals:

Preparer: Jason McMillan

Date: 16JUL14

Reviewer: Dr. Maggie Bryans

Date: 18JUL14

- 1. Purpose:** Purify GFP using the BioLogic LP Chromatography System with a HIC column.
- 2. Scope:** Applies to purifying GFP using Macro-Prep Methyl HIC resin and the BioLogic LP system.
- 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
 - 4.2. Bio-Rad's Macro-Prep HIC Media Instruction Manual, LIT486
- 5. Definitions:**
 - 5.1. CV: Column Volume; $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$
 - 5.2. L = Length of column (meaning the height of the bead bed)
 - 5.3. HETP: Height Equivalent to Theoretical Plate; $HETP = L/N$
 - 5.4. $N = 5.54 (t_R/w_{1/2})^2$
 - 5.5. t_R : retention time
 - 5.6. $w_{1/2}$: peak width at half height
 - 5.7. h: Reduced Plate Height; $h = HETP/D_p$
 - 5.8. D_p : bead diameter
- 6. Precautions:** N/A
- 7. Materials:**
 - 7.1. Deionized Water
 - 7.2. Buffer A Equilibration (2M $(NH_4)_2SO_4$)
 - 7.3. Buffer B Elution (Low Ionic Solution, TE Buffer)
 - 7.4. 20% Ethanol
 - 7.5. MilliQ Water
 - 7.6. Container for waste fluid
 - 7.7. Kim Wipes
- 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. Select "COM1" and click "OK"
 - 8.5. Purge System with Buffer A and Zero the UV Monitor**
 - 8.5.1. Place each buffer line into a container filled with Buffer A (Equilibration Buffer).
 - 8.5.2. Attach the column inlet tube directly to the column outlet tube using the tubing connector.

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- 8.5.3. Press the MANUAL mode key.
 - 8.5.4. Select BUFFER.
 - 8.5.5. Select MIX.
 - 8.5.6. Type in 50% B.
 - 8.5.7. Select OK.
 - 8.5.8. Select PURGE.
 - 8.5.9. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
 - 8.5.9.1. While the system is running, zero the UV Monitor.
 - 8.5.9.1.1. Press the UV instrument key.
 - 8.5.9.1.2. Select ZERO.
 - 8.5.9.1.3. Verify that the absorbance changes to zero on the display panel of the controller.
 - 8.5.9.1.4. Press the PUMP instrument key.
 - 8.5.10. After conductivity stabilizes, select STOP.
- 8.6. Attach the Column**
- 8.6.1. Position the valve to close off the column (silver handle is horizontal).
 - 8.6.2. Disconnect the column inlet and outlet tubing from the tubing connector.
 - 8.6.3. Attach the column inlet tubing from the injector valve to the top of the column.
 - 8.6.4. Attach the column outlet tubing to the bottom of the column.
 - 8.6.5. Open the valve at the bottom of the column (silver handle in vertical position).
 - 8.6.6. Press MANUAL mode key.
 - 8.6.7. Select PURGE.
 - 8.6.8. Allow buffer to drip into the waste container from the side port until air bubbles are completely absent from the tubing.
 - 8.6.9. Simultaneously select STOP.
 - 8.6.10. Place all lines in the appropriate buffers/solutions
- 8.7. Prepare the Sample**
- 8.7.1. Add 400µl of binding buffer to a micro centrifuge tube.
 - 8.7.2. Add 400µl of cell lysate to the same micro centrifuge tube. Mix gently by pipetting up and down until mixed properly.
- 8.8. Run the Column**
- 8.8.1. Turn the MV-6 injector valve knob counterclockwise until there is resistance.
 - 8.8.2. Draw 800µl of sample into a syringe.
 - 8.8.3. Insert the syringe into top port. Push slowly to fill sample loop while simultaneously collecting overflow in a beaker.
 - 8.8.4. Leave syringe in port.
 - 8.8.5. Press the PROGRAM mode key.
 - 8.8.6. Select LIST METHODS.
 - 8.8.7. Using the arrow keys, select the **GFPHIC** method.
 - 8.8.8. Select OPEN.
 - 8.8.9. Press the “Run” mode key.
 - 8.8.10. System will have a 10 second delay. The method will start.

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- 8.8.11. Turn the MV-6 injector valve knob clockwise until there is resistance.
- 8.8.12. Verify that the computer is recording data by the appearance of an S symbol on the graph.
 - 8.8.12.1. If the S symbol is not present, click the “record” button on the toolbar on the computer screen.
- 8.8.13. When the alarm sounds, turn the MV-6 injector valve knob counterclockwise until there is resistance.

8.9. Clean and Store the System

- 8.9.1. If the system will be used again with the same column within a few days, it may be stored “as is” after a run.
 - 8.9.1.1. Turn off the system and turn off the computer.
- 8.9.2. If the system will not be used within a few days it must be flushed with water then 20% ethanol and purged with air.
 - 8.9.2.1. Disconnect the column.
 - 8.9.2.2. Attach the column inlet tube directly to the column outlet tube.
 - 8.9.2.3. Place each buffer line into a container filled with MilliQ water.
 - 8.9.2.4. Attach the column inlet tube directly to the column outlet tube.
 - 8.9.2.5. Press the MANUAL mode key.
 - 8.9.2.6. Select BUFFER, then select MIX.
 - 8.9.2.7. Type in 50% B, then select OK.
 - 8.9.2.8. Select PURGE.
 - 8.9.2.9. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
 - 8.9.2.10. Select STOP.
 - 8.9.2.11. Place each buffer line into 20% ethanol and repeat steps 8.13.2.6. through 8.13.2.16.
 - 8.9.2.12. Place each buffer line on a lab towel or kimwipes so that they are open to the air and repeat steps 8.13.2.6. through 8.13.2.16.
 - 8.9.2.13. Place lines in 20% ethanol for storage.
 - 8.9.2.14. Turn off the LP Biologic System.

9. Attachments:

- 9.1. Figure 1: Controller Front Panel
- 9.2. Figure 2: Controller Pump
- 9.3. Figure 3: LP Biologic System Parts
- 9.4. Figure 4: Column components
- 9.5. Figure 5: Chromatogram example for calculating HETP

10. History:

Name	Date	Amendment
Deb Audino	070105	Initial release
Deb Audino	110405	Removed purging the system with water and Buffer B prior to use.

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		Added the cleaning and storing section.
Deb Audino	17May06	Added the column components figure, added steps that were removed from the process SOPs.
Bob O'Brien	23Jan08	Added steps to clarify use of the 3 way valve.
Deb Audino	04Apr08	College name change
Kari Britt	03Aug10	Added to definitions and HETP sections. Added Figure 5. Made grammar and formatting edits as needed throughout the document. Removed references to programming SOP.
Jason McMillan	16JUL14	Modified for GFP

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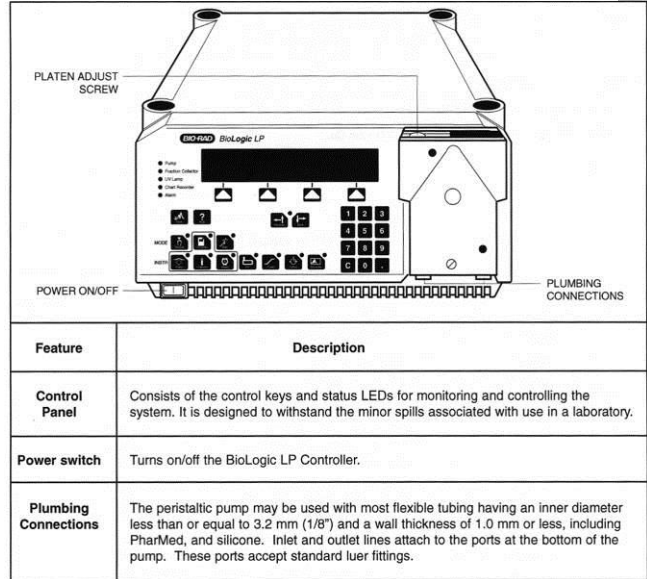


Figure 1: Controller Front Panel

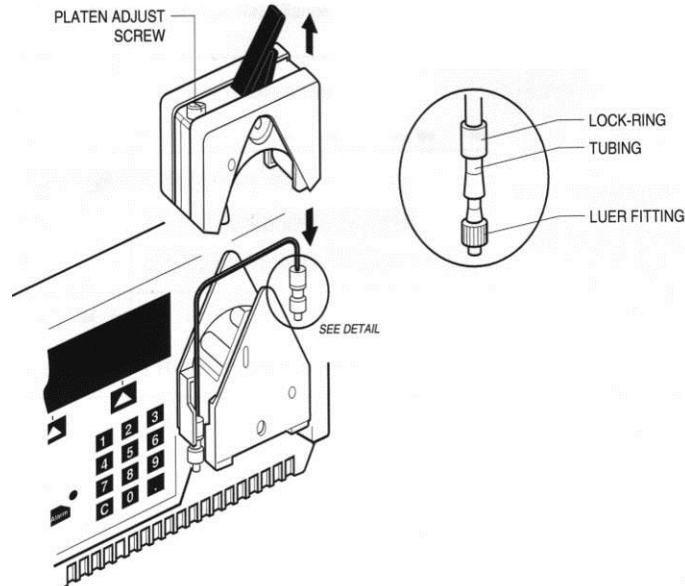


Figure 2: Controller Pump

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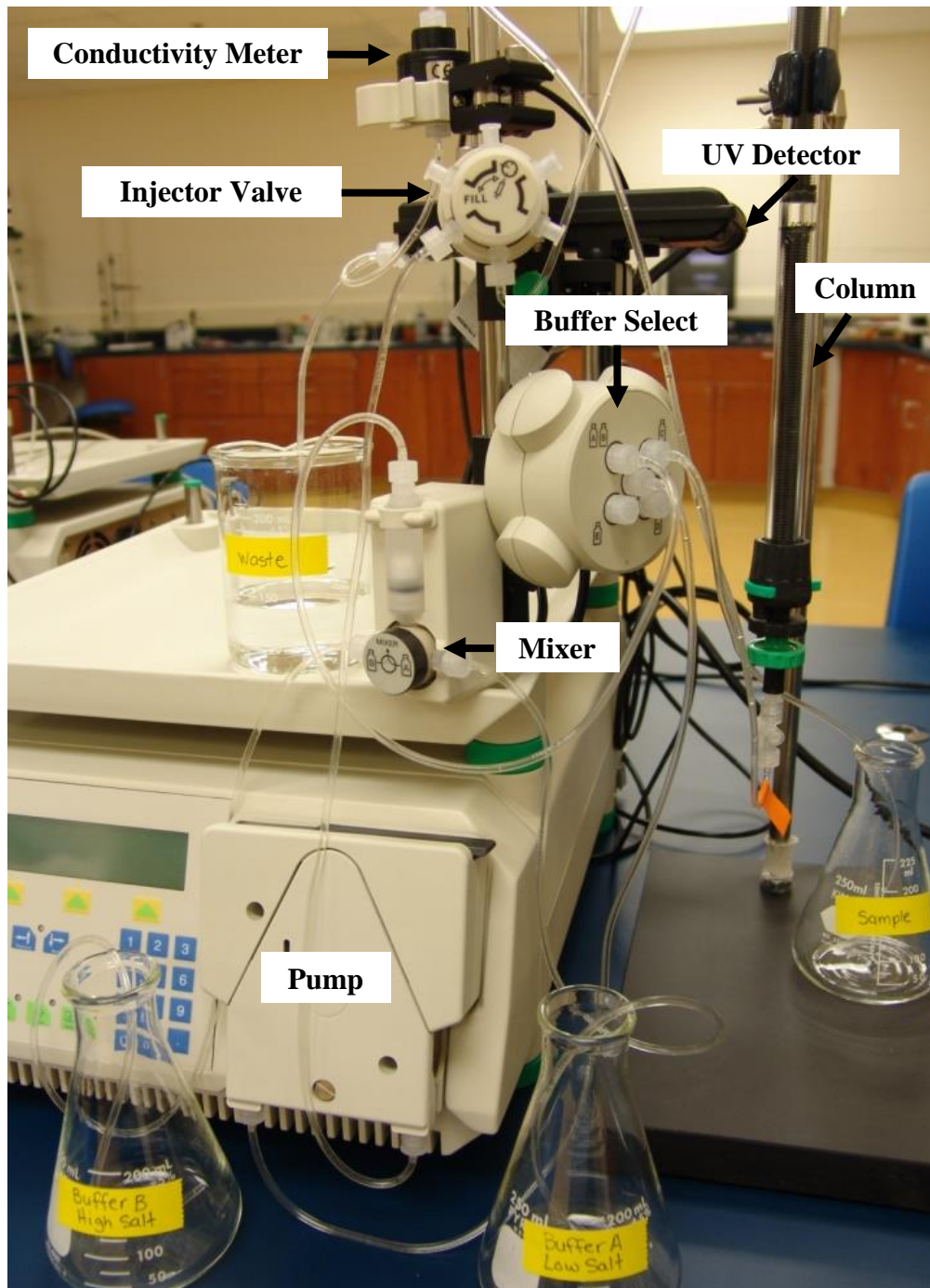


Figure 3: LP Biologic System Parts

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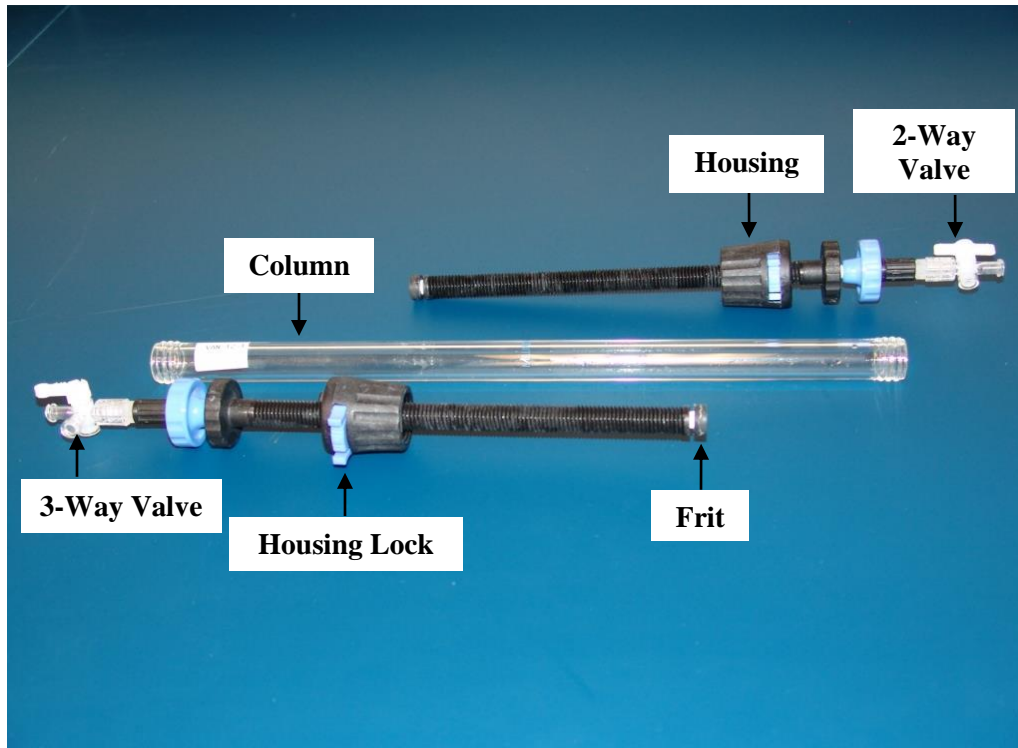


Figure 4: Column Components

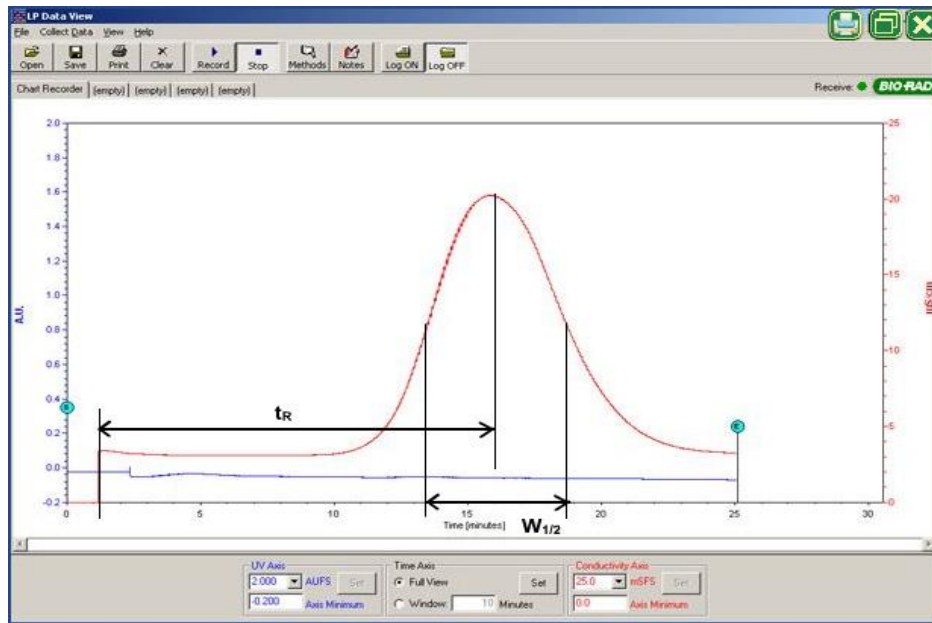


Figure 5: Chromatogram Example for Calculating HETP