Title: Anion Exchange Chromatography of GFP using the BioLogic LP Chromatography System

Approvals:

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1. **Purpose:**
   1.1. To perform an intermediate purification of GFP from bacteria cell lysates using the Biologic LP Chromatography System and LP DataView software.
   1.2. To purify small samples containing green fluorescent protein (GFP) by anion exchange chromatography using the Biologic LP Chromatography System and LP DataView software.

2. **Scope:**
   2.1. Applies to purification of GFP from diverse origins including bacterially expressed recombinant GFP from cell lysates that have been prepared using B-PER, (Bacterial Protein Extraction Reagent, thermos-Fisher) using a HiTrap Capto Q HP 5ml column installed on the Biologic LP chromatography System and LP DataView software.

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 (catalog numbers 731-8300, 731-8301)
   4.2. Biologic LP starter Kit Instruction Manual (catalog number 731-8350)
   4.3. Bio Frac Fraction Collector Instruction Manual
   4.4. Installation and Operation of LP Data View Software for the Biologic LP System
   4.5. GE Healthcare HiTrap Capto Q Ion Exchange Column (Instructions 11-0026-18 AF)
   4.6. SOP: BioLogic LP Chromatography System, Document Number: DP 7
   4.7. SOP: Anion Exchange Chromatography of Green Fluorescent Protein (GFP) using the AKTA Pure system, Document Number SU DP 20
5. **Definitions:**
   5.1. N/A
6. **Precautions:**
   6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
   6.2. Care must be taken to avoid air in the fluid path, which could damage the column or give spurious and uninterpretable readout from the UV and/or conductivity detectors.

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6.3. Buffers must be degassed and filtered prior to use with the Biologic LP Chromatography System instrument.

7. Materials:

7.1. Biologic LP Chromatography system
7.2. Bio Frac Fraction Collector
7.3. A computer with the LP Data View Software installed
7.4. GE healthcare HiTrap Capto Q 5ml column
7.5. Additional Lab Equipment: balance, table top centrifuge and rotor, pH meter
7.6. Lab Utensils: Beakers (250, 500ml, 1000 ml), 1 liter and 500 ml graduated cylinders
7.7. Lab Supplies: Filters (0.2μm) and bottles for vacuum filtration and degassing of all chromatography buffers, 1ml syringe, tubes for the fraction collector
7.8. Reagents:
  7.8.1. TRIS Base
  7.8.2. Sodium Chloride (RNAase and Protease free)
  7.8.3. 1 M HCL
  7.8.4. Filtered deionized water (MilliQ or similar).
  7.8.5. 20% Ethanol
7.9. Lab Supplies:
  7.9.1. Tubes for fraction collector (43)
  7.9.2. Lab film
  7.9.3. Paper towels

8. Procedure:

8.1. **Prepare buffers and solutions**
  8.1.1. Buffer A: Anion exchange Buffer: 20 mM Tris-HCl, pH 8.0
    8.1.1.1. Dissolve 2.423 gm Tris Base in 950 ml MilliQ water in a one-liter beaker with a stir bar.
    8.1.1.2. Titrate the pH of the Tris solution to pH 8.0 by addition of 3M HCL or 1M HCl, by carefully adding the acid dropwise.
    8.1.1.3. Adjust the final volume to 1 liter. Transfer 500ml of this solution to another beaker and Label the solution "Buffer A" along with its composition and date prepared. Filter and degas the buffer by passage through a vacuum filter device attached to house vacuum, leaving the filtered solution under vacuum for 15-20 minutes.
  8.1.2. Buffer B: 20 mM Tris-HCl, pH 8.0, 1M NaCl
    8.1.2.1. Use the remaining 500ml of Tris-HCl pH 8.0 solution to prepare Buffer B by dissolving 29.22g of NaCl in the remaining 500ml. Filter and degas this buffer and label the bottle "Buffer B" along with composition and preparation date.
    8.1.2.2.Filter and degas 500ml of MilliQ water.
  8.1.3. Filter and degas 250ml of 20% ethanol

8.2. **Sample Collection and Preparation**
  8.2.1. Preparation of bacterial cell lysate;
    8.2.1.1.Weigh an empty 2.0 ml microfuge tube and record the weight.

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8.2.1.2. Transfer approximately 150µg of bacterial cell pellet to the pre-weighed microfuge tube and determine the weight of cell pellet transferred.

8.2.1.3. Resuspend the pellet in a 1:10 ratio (w/v) of B-PER by pipetting up and down; use 1,500µl for 150µg of cell pellet.

8.2.1.4. Protease inhibitors can be added to this lysate if desired;

8.2.1.4.1. Add 3.8µl of a 0.2mg/ml solution of Leupeptin in MilliQ water.

8.2.1.4.2. Add 3µl of a 1.0 mg/ml solution of Aprotinin in MilliQ water.

8.2.1.4.3. Add 6µl of a 10mg/ml solution of PMSF in isopropanol.

8.2.1.5. Vortex for 10 seconds.

8.2.1.6. Shake for 10 minutes at room temperature on a multi wrist shaker.

8.2.1.7. Centrifuge the lysate at 15,000 rpm for 10 minutes at room temperature.

8.2.1.8. Transfer the supernatant to a fresh microtube.

8.2.1.9. Sterile filter the supernatant using a 0.2µm syringe top filter and store on ice until needed.

8.2.1.10. Save a 30µl aliquot in a tube labeled "pre-column" on ice for later analysis.

8.2.1.11. A volume of 0.6 ml of sample is needed for each injection.

8.2.2. Other samples containing GFP should be in a compatible buffer such as 20mM Tris-HCl, pH 8. A volume of 0.6 ml of sample is needed for each injection.

8.3. **Start-up and preparation of Biologic LP Instrument and computer**:

Degassed buffers should be in place prior to turning on the Biologic LP instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.

8.3.1. Place the degassed buffers A and B on top of the Biologic LP instrument.

8.3.2. Locate Inlet tubing A and B (atop the instrument, resting in water or 20% ethanol). **Note:** if the tubing is currently in 20% Ethanol the system will need to be flushed with filtered and degassed MilliQ water before any buffers. Refer to SOP DP76.

8.3.3. Transfer tubing Inlet A to the buffer A bottle.

8.3.4. Transfer tubing Inlet B to the buffer B bottle.

8.3.5. Place all 'Waste' tubes into collection beakers.

8.3.6. Place the Injection Loop overflow tube into a waste beaker.

8.3.7. The ON/Off switch for the Biologics System in located on the lower left front. Switch to ON.

8.3.8. Prepare the fraction collector for later steps by filling the carousel with clean tubes (17).

8.3.9. The On/Off switch for the fraction collector is located on the back of the collector next to the power cord. Switch to the 'On' position.

8.3.10. Turn on the computer.

8.3.11. Login to the computer using credentials provided by the College. Open the LP Data View Software.

8.3.12. **Purge the system**

8.3.12.1. Connect the column inlet tube directly to the column outlet tube using the tubing connector.

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8.3.12.2. Press the "Manual" mode key, note the conductivity and UV absorbance values are displayed on the Manual screen.
8.3.12.3. Press the Pump key
8.3.12.4. Press the Buffer key, use Previous/Next keys to select Buffer B, press OK.
8.3.12.5. Press Purge key. Purge the system with Buffer B until there is no air in the lines and the conductivity reading is stable. (less than 5 minutes)
8.3.12.6. Press the Buffer key, use the Previous/Next keys to select Buffer A, press OK. Purge the system with Buffer A until there is no air in the lines and the conductivity reading is stable.
8.3.12.7. Press the Stop key. (less than 5 minutes)

8.3.13. **Zero the UV monitor**
8.3.13.1. In Manual mode, with Buffer A selected, and the Pump selected;
8.3.13.2. Press the Flow soft key, set flow to 1.5 ml/min, Press OK, Press Start.
8.3.13.3. Press the UV soft key, Press SET RANGE.
8.3.13.4. Use the Increase/Decrease soft keys to set the UV monitor range to 0.05AUFS
8.3.13.5. Press OK
8.3.13.6. Press Zero
8.3.13.7. Press the Pump soft key
8.3.13.8. Press Stop

8.3.14. **Attach the column** (GE HiTrap Capto Q 5 ml) Refer to the BioLogic LP Chromatography System Operating SOP, Document Number DP 7 for directions regarding connecting the column.
8.3.14.1. Place the column on the system rack in a position convenient for connecting inlet and outlet tubing.
8.3.14.2. In Manual mode, Press Pump soft key, press Flow, set flow to 0.5 ml/min of Buffer A. Press OK.
8.3.14.3. Press Start Use a 250 ml beaker to catch waste.
8.3.14.4. Disconnect the tubing bridging the column inlet and outlet, remove the plug from the column inlet, allow a few drops of Buffer to drip into the column inlet to ensure the absence of air. Begin to connect the column inlet, leave this connection loose enough for liquid to escape so that there is no buildup of pressure in the column.
8.3.14.5. Remove the plug from the bottom of the column and attach the outlet tubing to the column.
8.3.14.6. Tighten the column inlet connection.

8.3.15. **Equilibrate the column** (This step is not necessary if the column was equilibrated as part of a previous run.)
8.3.15.1. In Manual mode using Buffer A, Press Flow, set flow to 2.5 ml/min. Press OK.
8.3.15.2. Press Start. wash the column for 10 minutes (5 column volumes).
8.3.15.3. Press Stop.

8.3.16. **Flush the Injection Loop**
8.3.16.1. Check that the Sample Inject Valve is in the fill position, (turned to the left)
8.3.16.2. Fill a 1ml syringe with Buffer A, remove any air bubbles.

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8.3.16.3. Attach the syringe to the Inject Port and flush the sample loop.
8.3.16.4. Draw at least 800µl of sample solution into the syringe, remove any air bubbles.
8.3.16.5. Place the syringe in the Injection Port and slowly fill the loop, leave the syringe in the port.

8.4. **Perform a chromatography run**:
8.4.1. Press F1 (Engage) on the BioFrac fraction collector. Check that the drop head is over tube #1
8.4.2. Press the Program key
8.4.3. Select List of Methods softkey, using the arrow keys select the method with file name "GFP AEX06 18".
8.4.4. Select Open
8.4.5. Press the Run mode softkey. There is a 10 second delay before the system starts.
8.4.6. Press Record on the LP Data View software. If the software is recording there is a "S" symbol on the screen.
8.4.7. Turn the Injector valve to the right, the "inject position"
8.4.8. Monitor the computer screen for error messages or warnings. The BioLogic LP screen shows that the fraction collector is diverting flow to waste.
8.4.9. An audible alarm is set to remind the operator to close the injection port once the wash step is completed. When the alarm sounds turn the Injection Valve to the left, the "fill position".
8.4.10. Press the Alarm soft key to silence the alarm.
8.4.11. Observe that the fraction collector is now collecting fractions.
8.4.12. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs.
8.4.13. Remove tubes from the fraction collector and place in a rack for storage at 4ºC, awaiting further analysis. Cover the top of the tubes with lab film.

8.5. ***Equipment shut-down and short term (less than 3 days) storage***
8.5.1. After completion of the final separation of the day, transfer Inlet tubing A and B to a flask of degassed deionized water (250ml or greater). **Without introducing air into the lines**.
8.5.2. Refer to the Biologic LP Chromatography System Operating SOP, Document Number DP 7, to clean the lines.
8.5.3. Turn off the instrument.

8.6. **Equipment shut-down and long term (3 days or more) storage**
8.6.1. After completion of the System Short Term Storage method, transfer Inlet tubing A and B to a flask of degassed 20% ethanol (250ml or greater).
8.6.2. Refer to the Biologic LP Chromatography System Operating SOP, Document Number DP 7, to clean the lines.

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8.6.3. Remove the GE HiTrap Capto Q column from the instrument, following the instructions given in the Biologic LP Chromatography System Operating SOP, Document Number DP 7, taking care to avoid the introduction of air into the column. Store the column at 4°C to 30°C.

8.6.4. Turn off the Biologic LP instrument.

8.7. **Printing Your Chromatogram**

8.7.1. Under File, choose to Print (or Save as PDF to use a different printer).

9. Attachments:

9.1. Programed Pump Method

Method GFP AEX06 18

Step	**Buffer**	**ml/min**	**Volume**	**Time minutes**	**# of CVs**	
Load and Wash	100% A	1.0	0-15ml	15	3	No fractions collected
Alarm			*@15ml*			*close Injection port*
Fraction collection window			*33 - 45ml 1ml fractions*			
Elute	Gradient 0% to 100% Buffer B	2.5	15-55ml	16	8	1ml fractions collected
Regenerate Column	100% B	2.5	55-65ml	4	2	No fractions collected
	100% A	2.5	65–90ml	10	5	No fractions collected

10. History:

Revision Number	Effective Date	Preparer	Description of change
0	06/28/19	Robin Zuck	Initial release