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SOP: Isolation of Anti IL-8 mAb by Protein A Affinity Chromatography on the Bio-Rad Biologic LP Chromatography System

Approvals:

Preparer: Lara Dowland Date: 10JUN18
Reviewer: Cianna Cooper Date: 12JUN18

1. **Purpose:**
   1.1. To isolate anti IL-8 mAb from conditioned medium (produced by CHO cells expressing recombinant anit-IL-8) using ultrafiltration and Protein A affinity chromatography with the Biologic LP Chromatography System.
2. **Scope:**
   2.1. Applies to purification of mAb from prepared conditioned medium, which has been concentrated and it buffer exchanged by ultrafiltration.
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 (electronic)
   4.2. BioRad Bio-Scale Mini Affi-Prep Protein A column -1 ml information booklet (BioRad)
   4.3. Centricon 15 (30kDa cutoff) Concentrator Users Guide
5. **Definitions: 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. It is imperative that pumps never be allowed to run dry. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors.
   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. BioRad Bio-Scale Mini Affi-Prep Protein A column -1 ml column; stored at 4ºC- bring to room temperature prior to installation.
   7.3. Additional Lab Equipment: balance, table top centrifuge w/ swinging bucket rotor
   7.4. Lab Utensils: Beakers (250, 500ml, 1000 ml), 1 liter and 500 ml graduated cylinders
   7.5. Reagents:
      7.5.1. 20 mM sodium phosphate buffer, pH 7.0
      7.5.2. 0.1 M citric acid, pH 3.0
      7.5.3. 1 M Tris base

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7.5.4. Filtered deionized water (MilliQ or similar).
7.5.5. 20% Ethanol
7.5.6. 10N NaOH
7.5.7. Stock solutions of protease inhibitors: 10 mg/ml PMSF in isopropanol, 2 mg/ml leupeptin, 10 mg/ml aprotinin.

7.6. Lab Supplies:
7.6.1. Millipore Centricon 15 (30 kDa cutoff) centrifugal concentrators (2).
7.6.2. Filters (0.2 μm); (3 bottle top; 2 syringe mounted)
7.6.3. Corning bottles for vacuum filtration, degassing of all chromatography buffers.
7.6.4. Syringe (20 ml) – (2).
7.6.5. Tubes for fraction collector – (27)
7.6.6. Graduated cylinders: 1L, 250 ml, 100 ml
7.6.7. Beakers: 1L, 400 ml, 200 ml

8. Procedure:

8.1. **Prepare buffers and solutions**

8.1.1. Buffer A: Binding buffer: 20 mM sodium phosphate, pH 7.0
8.1.1.1. Weigh 0.80 ± 0.02 gm NaH_2PO_4 and transfer to a 1 L flask with magnetic stir bar.
8.1.1.2. Weigh 3.60 ± 0.02 gm $Na_2HPO_4 \bullet 7H_2O$ and transfer to the same flask.
8.1.1.3. Measure 980 ml deionized water in a graduated cylinder and add the water to the solids in the flask.
8.1.1.4. Stir until the solids have dissolved, then adjust the pH to 7.0.
8.1.1.5. Transfer to a 1L graduated cylinder and adjust the final volume to 1L.
8.1.1.6. Sterile filter the solution, allowing it to degas for 15-20 minutes. Label appropriately.

8.1.2. Buffer B: Elution buffer: 0.1M citric acid, pH 3.0
8.1.2.1. Weigh 3.84 gm citric acid in a 400 ml beaker with magnetic stir bar.
8.1.2.2. Dissolve in 180 ml deionized water.
8.1.2.3. Adjust the pH dropwise with 10N NaOH, to a final pH of 3.0
8.1.2.4. Transfer the solution to a 250 ml graduated cylinder. Adjust the final volume to 200 ml.
8.1.2.5. Filter the solution, allowing it to degas for 15 – 20 minutes.
8.1.2.6. Label appropriately.

8.1.3. 1M Tris base: added to fraction collector tubes to rapidly neutralize acid-eluted fractions from the protein A column.
8.1.3.1. Weigh 12.11 gm Tris base [tris(hydroxymethyl)aminomethane] into a plastic weigh boat and transfer to a 200 ml beaker with a stir bar.
8.1.3.2. Measure 85 ml deionized water in a graduated cylinder and transfer the water to the beaker containing Tris powder. Stir until dissolved.
8.1.3.3. Transfer the Tris solution quantitatively to a 100 ml graduated cylinder, rinsing the beaker with small aliquots of water, which are then added to the cylinder until a final volume of 100 ml is obtained.

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8.1.3.4. Filter the solution. Degassing is not necessary.

8.2. **Sample Collection and Preparation**

The operator will require up to10 ml of sample per sample injection (see below). Conditioned medium from a late log phase/early stationary phase culture of cells producing the desired mAb in a low IgG medium is an excellent source.

8.2.1. Transfer conditioned medium to centrifuge bottles; centrifuge at 1000 x g for 10 minutes at 4ºC.

8.2.2. Decant the supernatant into a beaker (for smaller volumes that are to be filtered with a syringe-mounted filter) or directly into a bottle top 0.2µm filter. Filter the supernatant into a 50 ml conical tube or bottle.

8.2.3. Transfer 15 ml of the filtered supernatant into each of two Centricon 15 (30kDa) ultrafiltration devices. Centrifuge in the table top centrifuge with swinging bucket rotor at 3500 x *g*, 4ºC for 25 minutes.

8.2.4. Remove the filter insert, pour off the filtrate in the bottom tube and return the insert to the tube. Add additional CM to fill the upper chamber and repeat centrifugation.

8.2.5. Repeat step 9.2.4 until sufficient quantity (50-75 ml of starting material) of the supernatant is concentrated.

8.2.6. Resuspend the concentrate in buffer A and repeat centrifugation.

8.2.7. Remove the concentrate with a 200µl or 1000µl pipette and place in a small beaker. Rinse the ultrafiltration membrane multiple times with 1 ml aliquots of buffer A (at room temperature) and combine the rinses with the concentrate in the beaker, until a volume of approximately 10 ml has been reached.

8.2.8. Mix the concentrate and rinses, then draw into a 10 ml syringe. Mount a 0.22µm filter on the syringe and pass the mAb-containing solution through it into a clean beaker.

8.2.9. The sample must be at room temperature prior to application to the column.

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 and C (atop the instrument and resting in water or 20% ethanol).

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. Transfer tubing Inlet C to the 15ml Falcon tube in a rack containing the sample to be purified.

8.3.6. The On/Off switch for the instrument is located on the lower left front. Switch to the ‘On’ position.

8.3.7. Turn on the computer.

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

8.3.9. Confirm that the correct column (BioRad Protein A-HP 1 ml) is attached to the system. If not, refer to SOP2.00 BioLogic LP Chromatography System Operating SOP for directions on connecting the column.

8.3.10. Prepare the fraction collector for later steps by filling the carousel with clean tubes (28). Add 200µl 1M Tris to the bottom of each tube – this serves to rapidly neutralize the acidic eluent (which destabilizes some antibodies).

8.3.11. Place all 'Waste' tubing into a 500ml Erlenmeyer flask.

8.3.12. Place the 15ml Falcon tube containing the sample to be purified in a rack on top of the platform.

8.3.12.1. Place Buffer line C into the falcon tube. This will serve to inject the sample rather than using a loop.

8.4. **Performing a chromatography run**:

8.4.1. Press the List of Methods softkey, select the method with file name "Anti IL8".

8.4.2. Press the Run mode softkey.

8.4.3. Press Record on the LP Data Veiw software.

8.4.4. Observe that the fraction collector is receiving drops.

8.4.5. Monitor the computer screen for error messages or warnings.

8.4.6. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs.

8.4.7. 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. The peak of absorbance at 280 nm which eluted with the low pH buffer B contains purified mAb, which will be examined by the QC Biochemistry Dept.

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, B and C to a flask of degassed deionized water (250ml or greater).

8.5.2. Refer to SOP2.00 Biologic LP Chromatography System Operating SOP 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, B and C to a flask of degassed 20% ethanol (250ml or greater).

8.6.2. Refer to SOP2.00 Biologic LP Chromatography System Operating SOP to clean the lines.

8.6.3. Remove the BioRad Protein A-HP column from the instrument and cap both ends, taking care to avoid introduction of air into the column. Store the column in a refrigerator.

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).

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9. **Attachments:** N/A

10. History:

Name	Date	Amendment
Lara Dowland	12Jun18	Initial Release