

Title: Affinity Chromatography of HSA

Approvals:

Preparer: _____ Kari Britt _____ Date _____ 02Jun09 _____
Reviewer: _____ Bob O'Brien _____ Date _____ 02Jun09 _____

1. Purpose:

1.1. To purify HSA using affinity chromatography.

2. Scope:

2.1. Applies to purifying HSA using Affi-Gel Blue beads 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. Affi-Gel Blue Manufacturer's Instructions
4.2. pH meter SOP
4.3. Amicon/Millipore column assembly SOP
4.4. BioLogic LP SOP

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:

- 6.1. 2.5mM NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. Harmful if swallowed or inhaled.

7. Materials:

- 7.1. Amicon Vantage-L Biochromatography column and accessories
7.2. Affi-Gel Blue Gel beads from BioRad (Catalog number: 153-7301)
7.3. BioRad BioLogic LP System
7.4. 0.22 μ m sterile filter units (Nalgene)
7.5. waste beakers
7.6. laboratory film, such as Parafilm
7.7. 1mL syringe
7.8. ring stand with clamps
7.9. biopure water
7.10. Equilibration Buffer A: 20mM Phosphate buffer, pH 7.1
7.10.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
7.10.2. $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (sodium phosphate dibasic, heptahydrate)

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- 7.11. Elution Buffer B: 20mM Phosphate buffer, pH 7.1, 1M NaCl
 - 7.11.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
 - 7.11.2. $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (sodium phosphate dibasic, heptahydrate)
 - 7.11.3. NaCl (sodium chloride)
- 7.12. Cleaning solution: 2.5mM NaOH (sodium hydroxide)

8. Procedure:

8.1. Prepare buffers and solutions

- 8.1.1. **Buffer A:** Equilibration Buffer, 20mM phosphate, pH 7.1
 - 8.1.1.1. Weigh out 0.80g NaH_2PO_4 and place into a 1L container.
 - 8.1.1.2. Weigh out 3.60g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and place into the 1L container with the NaH_2PO_4 .
 - 8.1.1.3. Using a 1L graduated cylinder, measure 1L of deionized water.
 - 8.1.1.4. Transfer water to the 1L flask.
 - 8.1.1.5. Add magnetic stir bar and stir to dissolve.
 - 8.1.1.6. Adjust pH to 7.1
 - 8.1.1.7. Filter Sterilize.
 - 8.1.1.8. Label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 7.1, Store Room Temperature, Dispose: Drain, [date], [group], [initials].
- 8.1.2. **Buffer B:** Elution Buffer, 20mM phosphate, pH 7.1, 1M NaCl
 - 8.1.2.1. Weigh 29.2 g NaCl and place into a 500mL container.
 - 8.1.2.2. Using a 1L graduated cylinder, measure 500mL 20mM Phosphate buffer, pH 7.1 and transfer to the 500mL container containing NaCl.
 - 8.1.2.3. Add magnetic stir bar to the container and stir to dissolve.
 - 8.1.2.4. Filter sterilize.
 - 8.1.2.5. Label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 7.1, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].
- 8.1.3. **Cleaning Solution:** 2.5mM NaOH
 - 8.1.3.1. Using a 1L graduated cylinder, measure 1L of deionized water.
 - 8.1.3.2. Transfer water to a 1L vessel.
 - 8.1.3.3. Weigh 0.1g of NaOH.
 - 8.1.3.4. Transfer the NaOH to the 1L vessel containing water.
 - 8.1.3.5. Add a magnetic stir bar and stir to dissolve.
 - 8.1.3.6. Filter sterilize.
 - 8.1.3.7. Label as: Cleaning Solution, 2.5mM NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].
- 8.1.4. **Buffer C:** Use the concentrated HSA in 20mM Phosphate buffer, pH 7.1 obtained during upstream processing of HSA followed by diafiltration using tangential flow filtration. Label as: Buffer C, Concentrated HSA in 20mM Phosphate buffer, pH 7.1, Store: 2-8°, Dispose: Drain, [date], [group], [initials].
Note: 20mL of Buffer C (concentrated HSA) will be loaded on to the column, so the volume of Buffer C in the vessel should be at least 30mL to prevent air from infiltrating the column.

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- 8.2. **Purge BioLogic LP Sytem with Buffer A and zero the UV monitor per the Biologic LP Chromatography System SOP.**
- 8.3. **Pour Column per the BioLogic LP Chromatography System SOP.**
 - 8.3.1. Use approximately 5mL of Affi-Gel Blue Gel beads.
- 8.4. **Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP.**
- 8.5. **Pack Column per the BioLogic LP Chromatography System SOP.**
 - 8.5.1. Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the vessel opening with a laboratory film, such as Parafilm.
 - 8.5.2. Use Method: Affi Pack
 - Step 1: 0 to 40min Buffer A 0.5mL/min
- 8.6. **Determine the HETP and h of the column per the BioLogic LP Chromatography System SOP.**
 - 8.6.1. Use Method Affi HETP:
 - Step 1: 0 to 30min Buffer A 0.5mL/min
 - 8.6.2. The Dp of the bead is 0.3mm.
 - 8.6.3. The expected HETP is approximately 0.6mm
 - 8.6.4. The h calculation should be less than 3. If h is greater than 3, the desired product may not bind the column efficiently. In this case it is best to re-pack the column.
- 8.7. **Run Column per the BioLogic LP Chromatography System SOP.**
 - 8.7.1. Place the lines for Buffers A, B, and C into the vessels containing the appropriate buffer. Cover the vessels with laboratory film.
 - 8.7.2. Use Method: Affi HSA
 - Step 1: 0 to 80min Buffer C 0.25mL/min
 - Step 2: 80 to 120min Buffer A 0.5mL/min
 - Step 3: 120 to 160min Buffer B 0.5mL/min
 - Step 4: 160 to 200min Buffer A 0.5mL/min
 - 8.7.3. Collect 1-5mL of the flow through fraction when the first A.U. peak begins to plateau (approximately 40 minutes into the run).
 - 8.7.4. Collect the entire elution fraction when the second A.U. peak BEGINS to appear (approximately 100 minutes into the run).
 - 8.7.5. Store fractions at 2-8°C for SDS PAGE analysis.
- 8.8. **Clean the Column per the BioLogic LP Chromatography System SOP.**
 - 8.8.1. Place the lines for Buffer A and B into the Cleaning Solution, 2.5mM NaOH.
 - 8.8.2. Use Method: Affi Clean
 - 8.8.2.1. Step 1: 0 to 40min Buffer 50% B 0.5mL/min
- 8.9. **Clean and Store the System per the BioLogic LP Chromatography System SOP.**
9. **Attachments: N/A**

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10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
SCP		Changed from Millipore LC100 system to manual pump system.
Deb Audino	7/2005	Changed from manual pump system to BioLogic LP system.
Deb Audino	08May06	Removed steps associated with equipment operation to simplify the process SOP.
Deb Audino	18Jan08	Decreased flowrate and run time on step 1 for Affi HSA program.
Deb Audino	04Apr08	College name change
Kari Britt	31May09	Added labeling directions, directions for covering vessels with laboratory film, and directions for placing buffer lines into the appropriate buffer. Also made general grammar and formatting edits as needed throughout the document.