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Purification of HIV Envelope Protein

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This is an "in house protocol" which contains some relevant info and some details that only apply to my lab. The protocol is written for protein produced in a hollow fiber cartridge. It should work for cells grown in tissue culture flasks but you will have to work out the binding capacity of the lectin column. Please feel free to call me if you have questions (301-496-4553).

Lectin derived from snowdrop bulbs (*Galanthus nivalis*) recognizes the terminal D-mannose groups found on the HIV envelope glycoprotein gp120. Because gp120 is one of few glycoproteins possessing such mannose groups, columns utilizing *Galanthus nivalis* lectin affinity chromatography offer an efficient means of concentrating recombinant gp120 from cell culture supernatant with few contaminants.

1. Pouring a lectin column:

   Attach a valve to the bottom of an empty chromatography column with a volume around 10 ml (Bio-Rad Laboratories). Close the valve and clamp the column in a vertical position over a 50 ml tube. Pour 4 ml of *Galanthus nivalis* lectin-bound agarose slurry (two-2 ml vials from Vector Laboratories, catalog #AL-1243) into the column into the column. Avoid the formation of air bubbles in the slurry as it settles into the column. Allow the slurry to settle.

   Next attach a flow adaptor to the column (Bio-Rad). To do so, first add sterile PBS pH 7.4 to the column until the level of liquid is in the reservoir above the column. Attach the flow adaptor to a peristaltic pump and begin slowly pumping PBS through the adaptor. Ensure that no air bubbles remain in the tubing. Then clip the flow adaptor to the column, open the valve, and lower the plunger into the column until it meets the liquid. Make sure that no air bubbles are trapped beneath the plunger and lower the plunger until it meets the top of the slurry. Tighten the cam at the top of the flow adaptor to fix the seal at the bottom of the adaptor. Allow the peristaltic pump to continue and wash the column with 4 to 5 column volumes of PBS at rate of 0.2 ml/min.

2. Binding of gp120 to lectin:

   Using the peristaltic pump in 4^{0}C cold room, pump cell culture supernatant through the column at a rate of 0.2 ml/min. A volume of 1000 ml will typically saturate the lectin column. Collect the flow through if you wish to determine the efficiency of binding to the lectin. Wash the column with 15 ml of cold PBS.

3. Elution of gp120:

   Prepare 25 ml of 0.5 M methyl alpha-D-manno-pyranoside (Sigma Chemical Co.) in PBS and chill to 4^{0}C. Elute proteins bound to the lectin column by passing the mannose solution over the lectin column at a rate of 0.2 ml/min. Using a fraction collector and UV detector, collect, identify, and save the fractions containing protein. I have found 0.5 ml fractions most convenient. The expected purity of the protein is >70%.

4. Removal of contaminating proteins from eluted fractions through metal-affinity chromatography:

   Prepare a 1 ml Hi-Trap cobalt chelating column (Amersham-Pharmacia Biotech) by equilibrating it in PBS and then charging it with 2 ml 200 mM cobalt chloride using the superloop. Wash the excess cobalt with 10 ml PBS. Then pool the eluted fractions from the lectin column and pass them over the chelating column at a rate no greater than 0.5 ml/min. I often use rate of 0.2 ml/min. This will negatively select for gp120 by binding the contaminant proteins but allowing the gp120 to pass through. Collect the flow-through in 0.5 ml fractions using the fraction collector and UV detector.

5. Removal of mannose and remaining contaminant proteins through size exclusion chromatography:

   Use a standard desalting column, or alternatively, we use a "10 cm hemi-size exclusion column" that resolves any remaining proteins bellow 30 kD.

   Pour or purchase a Sephadex G-75 size exclusion column. Pool the fractions containing protein and run them over the Sephadex column at 0.2 ml/min. Collect the fractions containing protein in 0.5 ml volumes and concentrate them if necessary using a centricon filter.

6. Post-Binding column maintenance:

   Rinse lectin column with 4-5 times column volume of PBS.

   * Purified protein may be tested for endotoxin levels using commercially available assay kits.

Reference

Jones et al., *Vaccine* Vol **13** (11):991-999, 1995.