Lectin affinity chromatography

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This protocol originated from Affinity Chromatography: Principles and Methods (GE Healthcare Life Sciences). Glycoproteins and polysaccharides react reversibly, via specific sugar residues, with a group of proteins known as lectins. As ligands for purification media, lectins are used to isolate and separate glycoproteins, glycolipids, polysaccharides, subcellular particles and cells, and to purify detergent solubilized cell membrane components. Substances bound to the lectin are resolved by using a gradient of ionic strength or of a competitive binding substance.

Table 1. Specificity of lectins

Lectin	Specificity
Con A (Concanavalin A)	High-mannose type, branched α-mannosidic structures
WGA (Wheat germ agglutinin)	N-acetylglucosamine Chitin oligomer Sialic acid
JAC (Jacalin)	Galactosyl (β-1,3) N-acetylgalactosamine
SNA, EBL (Sambucus nigra lectin)	Sialic acid attached to terminal galactose in (β-2,6)
PNA (Peanut agglutinin)	Galactosyl (β-1,3) N-acetylgalactosamine (T-Antigen)
LCA (Lens culinaris agglutinin)	α-Linked mannose residues
PHA-L (Phaseolus vulgaris leucoagglutinin)	Tri/tetra-antennary complex-type N-glycan
AAL (Aleuria aurantia lectin)	Fucose linked (α-1,6) to N-acetylglucosamine

Protocol for Concanavalin A (Con A) lectin affinity chromatography

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Additional materials required

- Binding buffer : 20 mM Tris-HCl, 0.5 M NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.4
- Elution buffer : 0.1–0.5 M methyl-a-D-glucopyranoside (methyl-a-D-glucoside) or methyl-a-D-mannopyranoside (methyl-a-D-mannoside), 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4
- Column : Either gravity-flow or FPLC columns may be used.

Binding capacity of medium (Con A Sepharose 4B) : 20-45 mg/ml (porcine thyroglobulin)

Procedure

1. Pack the column (if required).

   ① Equilibrate all materials to the temperature at which the separation will be performed.

   ② Eliminate air by flushing column end pieces with the recommended buffer. Ensure no air is trapped under the column net. Close column outlet leaving 1–2 cm of buffer in the column.

   ③ Gently resuspend the medium.

   ④ Estimate the amount of slurry (resuspended medium) required on the basis of the recommendations supplied.

   ⑤ Pour the required volume of slurry into the column. Pouring down a glass rod held against the wall of the column will minimize the introduction of air bubbles.

   ⑥ Immediately fill the column with buffer.

   ⑦ Mount the column top piece and connect to a pump. (optional)

   ⑧ Open the column outlet and set the pump to the desired flow rate. (optional)

   ⑨ Maintain the packing flow rate for at least 3 column volumes after a constant bed height is obtained. Mark the bed height on the column.

⑩ Stop the pump and close the column outlet. Remove the top piece and carefully fill the rest of the column with buffer to form an upward meniscus at the top.

⑪ Insert the adaptor into the column at an angle, ensuring that no air is trapped under the net.

⑫ Slide the adaptor slowly down the column (the outlet of the adaptor should be open) until the mark is reached. Lock the adaptor in position.

⑬ Connect the column to the pump and begin equilibration. Re-position the adaptor if necessary.

2. Wash with at least 10 column volumes of binding buffer to remove preservative.
3. Equilibrate the column with 10 column volumes of binding buffer.
4. Apply the sample, using a low flow from 15 cm/h, during sample application (flow rate is the most significant factor to obtain maximum binding).
5. Wash with 5–10 column volumes of binding buffer or until no material appears in the eluent (monitored by UV absorption at A280 nm).
6. Elute with 5 column volumes of elution buffer.

- Recovery from Con A Sepharose 4B is decreased in the presence of detergents. If the glycoprotein of interest needs the presence of detergent and has affinity for either lentil lectin or wheat germ lectin, the media Lentil Lectin Sepharose 4B or Agarose Wheat Germ Lectin may provide a suitable alternative to improve recovery
- For complex samples containing glycoproteins with different affinities for the lectin, a continuous gradient or step elution may improve resolution. Recovery can sometimes be improved by pausing the flow for some minutes during elution.
- Elute tightly bound substances by lowering the pH. Note that elution below pH 4.0 is not recommended and that below pH 5.0 Mn^{2+} will begin to dissociate from the Con A and the column will need to be reloaded with Mn^{2+} before reuse.

Cleaning

Wash with 10 column volumes of 0.5 M NaCl, 20 mM Tris-HCl, pH 8.5, followed by 0.5 M NaCl, 20 mM acetate, pH 4.5. Repeat 3 times before re-equilibrating with binding buffer.
Remove strongly bound substances by:

- washing with 0.1 M borate, pH 6.5 at a low flow rate
- washing with 20% ethanol or up to 50% ethylene glycol
- washing with 0.1% Triton X-100 at 37 °C for one minute

Re-equilibrate immediately with 5 column volumes of binding buffer after any of these wash steps.

Protocol for Wheat Germ lectin affinity chromatography

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Additional materials required

- Binding buffer : 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4
- Elution buffer : 0.5 M N-acetylglucosamine, 20 mM Tris-HCl, pH 7.4
- Column : Either gravity-flow or FPLC columns may be used.

Procedure

1. Pack the column (if required) and wash with at least 10 column volumes of binding buffer to remove preservative.
2. Equilibrate the column with 10 column volumes of binding buffer.
3. Apply the sample, using a low flow from 15 cm/h, during sample application (flow rate is the most significant factor for maximum binding).
4. Wash with 5–10 column volumes of binding buffer or until no material appears in the eluent (monitored by UV absorption at A280 nm).
5. Elute with 5 column volumes of elution buffer.

- Use 0–0.5 M N-acetylglucosamine, 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4 with a continuous gradient or step elution to improve resolution of complex samples containing glycoproteins with different affinities for the lectin.
- Elute tightly bound substances with 20 mM acetate buffer, pH 4.5 or with an alternative sugar, for example triacetylchitotriose.
- Higher concentrations of eluting substances may be necessary and recovery may be improved by pausing the flow for some minutes during elution.

Cleaning

Wash with 5–10 column volumes of 20 mM Tris-HCl, 1 M NaCl, pH 8.5 and re-equilibrate immediately with binding buffer. Low concentrations of non-ionic detergents in the Tris-HCl buffer can be used if necessary, for example 0.1% Nonidet P-40.