instructions

Lentil Lectin Sepharose 4B

Lentil Lectin Sepharose™ 4B is lentil lectin coupled to Sepharose 4B by the cyanogen bromide method.

Lentil lectin is a metalloprotein, containing Ca^{2}+ and Mn^{2}+, which binds reversibly to poly-saccharides and glycoconjugates containing glucose or mannose type sugars. Lentil Lectin Sepharose 4B is a generally applicable group specific adsorbent and is routinely used in the preparation and purification of a number of glycoproteins and carbohydrate containing molecules. Detergent-solubilized membrane glycoproteins, cell surface antigens viral glycoproteins may also be purified using Lentil Lectin Sepharose 4B.

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Table 1. Medium characteristics.

Ligand density:	1.9–4.2 mg lentil lectin/ml drained medium
Binding capacity*:	>16 mg porcine thyroglobulin/ml medium
Bead structure:	4% agarose
Bead size range:	45–165 µm
Average particle size:	90 µm
Max linear flow rate**:	75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH stability***	
Long term:	3–10
Short term:	3–10
Chemical stability:	Stable to all commonly used aqueous buffers. Chelating agents such as EDTA, 8 M urea or solutions having a pH below 3 should be avoided as these conditions result in removal of manganese from the lectin and loss of activity.
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.
Storage:	20% ethanol

* The capacity data were determined in 0.1 M phosphate buffer pH 7.0.

** Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

*** The ranges given are estimates based on our knowledge and experience. Please note the following:

pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

pH stability, short term refers to the pH interval for regeneration and cleaning.

Preparing the medium

Lentil Lectin Sepharose 4B is supplied pre-swollen in 20% ethanol. Wash the required amount of medium with 10 volumes of binding buffer to remove the ethanol solution. Prepare a slurry with binding buffer, see below, in a ratio of 75% settled medium to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

Packing Sepharose 4B

1. Equilibrate all material to the temperature at which the chromatography will be performed.

2. De-gas the medium slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see Table 1, is typically employed during packing.

   Note: If you have packed at the maximum linear flow rate, do not exceed 75% of this in subsequent chromatographic procedures.
7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adaptor

Adaptors should be fitted as follows:

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.

3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the medium bed is stable. Re-position the adaptor on the medium surface as necessary.

The column is now equilibrated and ready for use.

Binding

Binding of glycoproteins and carbohydrate containing proteins occurs at neutral pH. The binding of substances to Lentil Lectin Sepharose 4B requires the presence of both Mn^{2}+ and Ca^{2}+. These are present in large excess in the medium supplied. The protein-metal ion complex remains active and is stable at neutral pH even in the absence of the free metal ions. However, to preserve the binding activity below pH 5, excess Mn^{2}+ and Ca^{2}+ (1 mM) must be present. Recommended binding buffer is 20 mM Tris-HCl, pH 7.4 containing 0.5 M NaCl to avoid non-specific ionic interactions.

Elution

Elution of bound substances can be achieved using an increasing gradient (continous or step) of α-D-methylmannoside or α-D-methylglucoside. These sugars act as strong eluents. Many substances elute at 0.1–0.2 M but higher concentrations may be reguired for more tightly bound substances. Glucose and mannose may also be used but are weaker eluents.

Strongly bound substances may also be eluted using low pH, not below pH 3, or with a borate buffer, 0.1 M pH 6.5. Elution of strongly bound substances may be facilitated by including 1% deoxycholate, or other detergent, in the elution buffer.

Regeneration

Lentil Lectin Sepharose 4B may be regenerated for re-use by washing the medium alternatively with 2–3 bed volumes of high pH (8.5) and low pH (5.5) buffer solutions containing 0.5 M NaCl. This cycle should be repeated 3 times followed by re-equilibration with 3–5 bed volumes of binding buffer.
All strongly bound substances may not have been eluted during the regeneration procedure. In difficult cases use a detergent (0.1% non-ionic) borate buffer at low flow rate. A 20% ethanol wash or a gradient wash with up to 50% ethylene glycol may be used to elute even the most strongly bound substances.

An alternative method for regeneration is to wash the medium with a detergent solution, *e.g.* 0.1% Triton™ X-100 at 37 °C for one minute. Re-equilibrate immediately with at least 5 bed volumes of binding buffer.

Storage

Lentil Lectin Sepharose 4B should be stored at 4–8 °C in 20% ethanol.

Further information

Check www.chromatography.amershambiosciences.com for more information. Useful information is also available in the Affinity Chromatography Handbook, see ordering information.

Ordering information

Product	Pack size	Code No.
Lentil Lectin Sepharose 4B	25 ml	17-0444-01

Litterature

Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

Trademarks

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