Lentil Lectin Sepharose 4B resin for glycoprotein purification

Product Information

Cat#No#	Le-373C

Product Overview

Lentil Lectin Sepharose 4B resin is used for glycoprotein purification and purification of other carbohydrate-containing molecules. Binds molecules that contain α-D-mannose, α-D-glucose, and sterically related sugars. Compared to Con A, lentil lectin distinguishes less sharply between glucosyl and mannosyl residues. Retains binding in 1% deoxycholate and is valuable for purifying detergent-solubilized membrane proteins.

Description

Lentil lectin is a metalloprotein, containing Ca2+ and Mn2+, which binds reversibly to polysaccharides 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.

Applications

For purification, isolation or removal of molecules containing branched mannoses with fucose linked α(1,6) to the N-acetylglucosamine, (αMan>αGlc>GlcNAc) and sterically related residues like glycoproteins, membrane proteins, glycolipids, lipoproteins, pol.

Maximum operating pressure

Base matrix 70-140 cm/h, pressure drop cm H2O/bed height=15, bed height 10 cm, 5 cm i.d.

Medium Preparation

Lentil Lectin Sepharose 4B is supplied preswollen in 20% ethanol containing 0.9% NaCl, 1 mM CaCl2 and 1 mM MnCl2 . Wash the required amount of medium with 10 volumes of binding buffer to remove the ethanol solution. Prepare a slurry with binding buffer 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.

Ligand Coupling Method

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CREATIVE BIOMART®
Chromatography

Lentil Lectin Sepharose 4B resin for glycoprotein purification

Cyanogen bromide activation
Matrix
Agarose, 4%
Particle Size
45 µm-165 µm
Average particle size
~90 µm
Ligand
Lentil lectin
Ligand density
2 mg Lentil lectin/ml medium
Recommended flow rate
< 75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
Recommended column height
25 cm
Chemical stability
Stable to 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.
pH working range
3–10
CIP stability

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3–10

Storage

2 to 8°C, 20% Ethanol, 0.9% NaCl, 1 mM Ca2+ and 1 mM Mn2+

Binding buffer

20 mM Tris-HCl, pH 7.4 containing 0.5 M NaCl

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 Mn2+ and Ca2+. 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 Ca2+ (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-Dmethylmannoside or α-D-methylglucoside. These sugars act as strong eluents. Many substances elute at 0.1 to 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 to 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 reequilibration with 3 to 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

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X-100 at 37 °C for one minute. Re-equilibrate immediately with at least 5 bed volumes of binding buffer.

Pack size
25 mL

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