HiTrap Con A 4B Columns

Product Information

Cat#No#	Hi-074P

Product Overview

HiTrap Con A 4B is a ready-to-use column prepacked with Con A Sepharose 4B, a resin for convenient separation and purification of glycoproteins, polysaccharides, and glycolipids.

Description

Concanavalin A (Con A) is a tetrameric metalloprotein isolated from Canavalia ensiformis (jack bean). Con A binds molecules containing α-D-mannopyranosyl, α-D-glucopyranosyl, and sterically related residues. The binding sugar requires the presence of C-3, C-4 and C-5 hydroxyl groups for reaction with Con A. Con A coupled to Sepharose is routinely used for separation and purification of glycoproteins, polysaccharides, and glycolipids.

Maximum operating pressure

5 bar [0.5 MPa] (70 psi)

Matrix

4% agarose

Average particle size

~ 90 µm

Ligand

Concanavalin A

Ligand density

10 to 15 mg Con A/mL resin

Dynamic binding capacity

20 to 45 mg porcine thyroglobulin/mL resin.

Recommended flow rate

0.1 to 1 mL/min (1 mL); 0.5 to 5 mL/min (5 mL).

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CREATIVE BIOMART®
Chromatography

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Recommended column height
25 mm
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 results in removal of manganese from the lectin with loss of activity as a result.
pH working range
4 to 9
Storage
4 to 8°C, 20% Ethanol containing 0.1 M Acetate Buffer pH 6, 1 M NaCl, 1 mM CaCl2, 1 mM MnCl2 and 1 mM MgCl2.
Binding buffer
20 mM Tris-HCl, 0.5 M NaCl, 1 mM MnCl2, 1 mM CaCl2, pH 7.4.
Elution buffer
0.1 to 0.5 M methyl-α-D-glucopyranoside (methylα-D-glucoside) or methyl-α-D-mannopyranoside (methyl-α-D-mannoside), 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4.
Binding
Binding of glycoproteins and carbohydrate containing proteins occurs at neutral pH. The binding of substances to Con A Sepharose 4B requires the presence of both Mn2+ and Ca2+. 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 of the Con A molecule below pH 5, excess Mn2+ and Ca2+ (1 mM) must be present. This will ensure an active Con A-metal complex.
Elution
Elution of bound substances can be achieved using an increasing gradient (linear or step) of methyl-α-Dmannopyranoside (methyl-α-D-mannoside) or methyl-α-Dglucopyranoside (methyl-α-D-glucoside). These sugars act as strong eluents. Many substances elute at 0.1 to 0.2 M but higher concentrations might be

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required for more tightly bound substances. Glucose and mannose may also be used but are weaker eluents. The recovery of glycoproteins can sometimes be improved by pausing the flow for a couple of minutes during elution. Tightly bound substances can also be eluted by lowering the pH, but not below pH 4.
Borate is known to form complexes with cis-diols on sugar residues and thus act as an competing eluent. For elution with borate, use a 0.1 M borate buffer, pH 6.5. Recovery on HiTrap Con A 4B is decreased in the presence of detergents.

Scaling up

1. Fill the syringe or pump tubing with binding buffer.
2. Remove the stopper and connect the column to the syringe or pump tubing—“drop-to-drop”—to avoid introducing air into the column. Use the provided luer adapter for the syringe.
3. Remove the snap-off end at the column outlet. Wash out the storage solution with 5 to 10 CV of distilled water or binding buffer. 4. Equilibrate the column with at least 10 CV binding buffer at 1 mL/min or 5 mL/min for 1 mL and 5 mL columns respectively to make sure that unbound Con A has been removed.
5. Apply the sample using a syringe fitted to the luer adapter or by pumping it onto the column. Use low flow rates: 0.1 to 0.5 mL/min, or 0.5 to 2.5 mL/min for 1 mL and 5 mL columns respectively.
6. Wash with 5 to 10 CV binding buffer or until no material appears in the effluent.
7. Elute with 5 CV elution buffer. The eluted fractions can be buffer exchanged using a HiTrap Desalting, HiPrep 26/10 Desalting, or Desalting PD-10 column.

Pack size

5 × 1 mL

Maximum flow velocity

1 mL/min (1 mL); 5 mL/min (5 mL).

Dimensions

7 × 25 mm

Column volume

1 mL

Column i.d.

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7 mm
Column hardware pressure limit
0.5 MPa (5 bar)

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