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Product Information

Concanavalin A-Sepharose® 4B

Catalog Number **C9017**
Storage Temperature 2–8 °C
Exact replacement for Catalog Number 27700

Product Description

Concanavalin A (Con A) is a lectin isolated from the jack bean. At pH >7 it exists as a tetramer.[1] This is the species most likely attached to the resin. Con A is not a glycoprotein.[3] It does not contain cysteine residues.[4] Unlike most other lectins, Con A is a metalloprotein and requires a transition metal ion, such as manganese and calcium ions for binding.[2] Each subunit of Con A binds one calcium ion and one manganese ion. Removal of these cations under acidic conditions abolishes the carbohydrate-binding activity.[5]

Con A binds specifically to mannosyl and glucosyl residues of polysaccharides and glycoproteins.[5] Unmodified hydroxyl groups at the C3, C4, and C6 positions of D-glucopyranosyl or D-mannopyranosyl rings may be essential for binding.[6]

General adsorption and desorption procedures and methods to prevent leakage of the lectin from the matrix have been described.[7–9] This product has been used to isolate human fibroblast interferon[10] and glycoprotein from sarcoplasmic reticulum.[11]

Con A Sepharose has been used with SDS (0.05%)[12] and Triton™ X-100.[13]

This product is supplied in 0.1 M acetate buffer, pH 6, containing 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 1 mM $MgCl_2$ in 20% ethanol as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedures

Since Con A requires Mn^{2+} and Ca^{2+} ions for carbohydrate binding, buffers should either include these metal ions or the the gel should be equilibrated with these ions, immediately prior to use. This is particularly important if the sample may contain traces of chelating agent or if the resin had been treated under acidic conditions (pH <5).

The procedure should be conducted at 2–8 °C.

1. Pre-wash column with 5 column volumes of wash solution (1 M NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, and 5 mM $CaCl_2$).
2. Equilibrate column in the buffer of choice (pH ranges generally between 6.5–7.5 although buffers as low as pH 4.1 and as high as 9.0 have been used successfully). A commonly used starting buffer is 20 mM Tris, pH 7.4, containing 0.5 M NaCl.
3. Load sample solution in equilibration buffer (protein concentrations 1–20 mg/ml, free of particulates).
4. Wash the resin with equilibration buffer until eluent solution is protein free.

Elution:

5. Elute the target protein with gradient or step-wise elution with methyl α-D-glucopyranoside or methyl α-D-mannopyranoside, glucose, or mannose (5–500 mM).
6. Maximum recovery and cleaning of the resin may be achieved by using 1 M sucrose, glucose, mannose, or corresponding α-methyl glysoside. The addition of chaotropic agents (0.5–6 M) may also be required for maximum recovery, but these denaturing conditions may severely damage the resin. Therefore they should be used last.

Regeneration:

7. After use, wash the column with 5–10 column volumes of 0.1 M borate, pH 8.5, or 0.1 M Tris, pH 8.5, containing 0.5 M NaCl.
8. This should be followed by 0.1 M sodium acetate, pH 4.5, containing 1.0 M NaCl.
9. The acid and basic washes may need to be cycled 2–3 times for complete washing.
10. Finally, the gel should be stored at 2–8 °C in 1.0 M NaCl containing 5 mM $CaCl_2$, 5 mM $MgCl_2$, and 5 mM $MnCl_2$ or in 0.1 M acetate buffer, pH 6, containing 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 1 mM $MgCl_2$, with 20% ethanol as a preservative.

References

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