

Concanavalin A Agarose (Kit No. CONA-5, CONA-10)

Concanavalin A is a globular protein isolated from jack beans with specific saccharide binding properties. This haemagglutinin is widely used in biochemical research. Concanavalin A will bind to α -D-glucose residues internal as well as terminal.

Immobilized Concanavalin A can be used for glycoprotein, polysaccharide and glycopeptide purification with Immunoglobulins being an area of major interest.

Procedure: Note: Con A requires Mn^{+2} and Ca^{+2} for carbohydrate coupling. Buffers used should either include these metal ions or the procedures should proceed immediately after replenishing ions through column pre-wash.

- 1) Pre-wash column with 5 column volumes of 1 M NaCl + 5 mM $MgCl_2$ + 5 mM $MnCl_2$ + 5 mM $CaCl_2$. Column volume = 2.5 mls packed gel.
- 2) Equilibrate column in the buffer of choice (pH ranges generally between 6.5 to 7.5 although buffers as low as pH 4.1 and as high as 9.0 have been used successfully). Detergents additives (i.e., Triton X-100, SDS, and deoxycholate) have also been used.
- 3) Procedures are generally conducted between 3-8°C.
- 4) Load sample (protein concentrations 1-20 mg/ml). Sample should be free of particulates, equivalent in pH and ionic strength as the column.
- 5) Wash into the column with small amounts of equilibration buffer (0.1-0.5 mls).
- 6) Wash columns with equilibration buffer until eluent solution is protein free.
- 7) Elute the target protein with gradient or step-wise elution. Eluting buffers may contain glucose, sucrose, methyl α -D-glucopyranoside or methyl α -D-mannopyranoside 5 mM to 100 mM. The additions of chaotropic agents (0.5 M to 6 M) may also be required for maximum recovery, but these denaturing conditions may severely damage the resin effecting reusability. The chaotropic agents should be considered a last resort.

Regeneration

- 1) After use, wash the column with 5-10 column volumes of 0.1 M Borate pH 8.5 followed by 0.1 M Sodium acetate pH 4.5 + 1.0 M NaCl, finally 1.0 M NaCl + 5 mM $CaCl_2$ + 5 mM $MgCl_2$ + 5 mM $MnCl_2$. Material can be stored in final buffer with 0.02% Thimerosal.
- 2) Note: "Stripping" of the column may also be necessary using high concentrations (up to 1 M) of competing carbohydrate.

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Storage: Store column upright with both caps in place at 3-8°C. 0.01 to 0.02% Thimerosal may be added for long term storage. **DO NOT FREEZE!**

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