

Glycoprotein Purification

Purification of proteins selectively utilizing their glycan component as a capture target is commonly done utilizing affinity chromatography. The most popular affinity matrices are m-aminophenylboronic acid agarose and the immobilized lectins, Con A and Wheat Germ.

m-Aminophenylboronic Acid Matrices

m-Aminophenylboronic acid matrices are capable of forming temporary bonds with any molecule that contains a 1,2-*cis*-diol group.

Procedure

- Equilibration buffers should be of low ionic strength, with pH 7-9.
- For a column volume of 1 ml, apply 1-2 mg of protein in approximately 250 μ l of buffer: 50 mM taurine/NaOH, pH 8.7, containing 20 mM $MgCl_2$.
- Optimize the column flow rate to 2 ml/hour, collecting 2 ml fractions.
- Elute the bound protein using the same buffer with 50 mM sorbitol or 50 mM Tris/HCl added.

References

1. Mallia, A.K. et al., *Anal. Letters*, **14 (B8)**, 649-661 (1981).
2. *Immobilized Affinity Ligand Techniques*, Hermanson, G.T., et al., Eds. (Academic Press, 1992), pp. 338, 339-392.
3. *Affinity Chromatography: A Practical Approach*, P.D.G Dean, W.S. Johnson and F. A. Middle, Eds., (IRL Press, 1985), p. 133.

Aminophenylboronate Matrices

A 8530	m-Aminophenylboronic Acid Affinity Medium	Matrix: cross-linked 6% beaded agarose Activation: epoxy, with attachment through the amino group, with a 12-atom spacer Ligand immobilized: 5-20 μ moles per ml Form: (light pink) suspension in 0.5 M NaCl, with 0.1 M sodium acetate, pH 5.0 Synonym: PBA-agarose
A 4046	m-Aminophenylboronic Acid Affinity Medium	Matrix: acrylic beads Activation: oxirane, with attachment through the amino group, with a 5-atom spacer Ligand immobilized: 300-600 μ moles per gram Form: lyophilized powder Swelling: 1 g swells to approximately 4 ml
A 8312	m-Aminophenylboronic Acid Affinity Medium	Matrix: 6% beaded agarose Activation: epichlorohydrin, with attachment through amino group with a 9-atom spacer Ligand immobilized: 40-90 μ moles per ml Binding Capacity: 8-14 mg Peroxidase Type VI per ml Form: suspension in water containing 0.002% chlorhexidine diacetate.

Lectin Matrices

(For additional Lectins see page 80)

Concanavalin A matrices bind specifically to mannosyl and glucosyl residues of polysaccharides and glycoproteins. Unmodified hydroxyl groups at the C3, C4, and C6 positions of D-glucopyranosyl or D-mannopyranosyl rings may be essential for binding. Con A matrices have been used with SDS (0.05%) and TRITON® X-100.

Procedure

Equilibration and Binding:

- Pre-wash column with 5 column volumes of wash solution (1 M NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, and 5 mM $CaCl_2$).
- 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). A commonly used starting buffer is 20 mM Tris, pH 7.4, containing 0.5 M NaCl.
- Load sample solution in equilibration buffer (protein concentrations 1-20 mg/ml, free of particulates).
- Wash the resin with equilibration buffer until eluent is protein free.

Elution:

- Elute the target protein with gradient or step-wise elution with methyl α -D-glucopyranoside or methyl α -D-mannopyranoside, glucose, or mannose (5 mM - 500 mM).
- Maximum recovery and cleaning of the resin may be achieved by using 1 M sucrose, glucose, mannose, or corresponding α -methyl glycoside. The addition 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. Therefore they should only be used as a last resort.

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Con A Matrices

C 9017	Concanavalin A Immobilized	Matrix: Sepharose 4B Activation: cyanogen bromide Ligand immobilized: 10-15 mg per ml Binding Capacity: 20-45 mg thyroglobulin per ml Form: suspension in 0.1 M acetate buffer, pH 6.0, containing 1 M NaCl, 1 mM each of CaCl ₂ , MgCl ₂ , and MnCl ₂ and 0.01% thimerosal
C 6170	Concanavalin A Immobilized	Matrix: 4% beaded agarose Activation: cyanogen bromide Ligand immobilized: approx. 15 mg per ml Binding Capacity: 6 mg yeast mannan per ml Form: suspension in 0.1 M acetate buffer, pH 6.0, containing 1 M NaCl, 1 mM each of CaCl ₂ , MgCl ₂ , and MnCl ₂ and 0.02% thimerosal

References

1. Yahara, I. and Edelman, G.M., Restriction of the mobility of lymphocyte immunoglobulin receptors by concanavalin A. *Proc. Nat. Acad. Sci. USA*, **69(3)**, 608-612 (1972).
2. Li, Y., et al., The p185^{neu}-containing Glycoprotein Complex of a Microfilament-associated Signal Transduction Particle. *J. Biol. Chem.*, **274(36)**, 25651-25658 (1999).
3. Romero, P.A., et al., Glycoprotein biosynthesis in *Saccharomyces cerevisiae*. Partial purification of the alpha-1,6-mannosyltransferase that initiates outer chain synthesis. *Glycobiology*, **4(2)**, 135-140 (1994).

Wheat Germ (*Triticum vulgare*) matrices are specific for GlcNAc₂ or NeuNAc residues.

Procedure

Equilibration and Binding:

- Wash and equilibrate column with 5 column volumes of wash solution (0.05 M sodium phosphate, pH 7.0, containing 0.2 M NaCl).
- Load sample solution in equilibration buffer (protein concentrations 1-20 mg/ml, free of particulates).
- Wash the resin with equilibration buffer until eluent is protein free.

Elution:

- Elute the target protein with gradient or step-wise elution with equilibration buffer containing 100 mg/ml N-acetylglucosamine (Product Code [A 8625](#)).

WGA Matrices

L 1882	Wheat Germ (<i>Triticum vulgare</i>) Lectin Immobilized	Matrix: Cross-Linked 4% beaded agarose Activation: cyanogen bromide Ligand immobilized: 5-10 mg per ml Form: Suspension in 1.0 M NaCl and 0.02% thimerosal
L 1394	Wheat Germ (<i>Triticum vulgare</i>) Lectin Immobilized	Matrix: 6% agarose macrobeads Activation: cyanogen bromide Ligand immobilized: Approx. 6 mg per ml Binding Capacity: 1-2 mg ovomucoid per ml Form: Suspension in 0.9% NaCl and 0.01% thimerosal

References

1. Lotan, R., et al., Activities of lectins and their immobilized derivatives in detergent solutions. Implications on the use of lectin affinity chromatography for the purification of membrane glycoproteins. *Biochem.*, **16**, 1787-1794 (1977).
2. Janicott, M., et al., The insulin-like growth factor 1 (IGF-1) receptor is responsible for mediating the effects of insulin, IGF-1, and IGF-2 in *Xenopus laevis* oocytes. *J. Biol. Chem.*, **266**, 9382 (1991).