3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

m-AMINOPHENYLBORONIC ACID
AFFINITY PRODUCTS
Sigma Prod. Nos. A4046, A8312, and A8530

PHYSICAL DESCRIPTION:[1]

A8530 Matrix: cross-linked 6% beaded agarose
Activation: epoxy, with attachment through the amino group, with a 12-atom spacer
Ligand immobilized: 5-20 μmoles per mL
Form: (light pink) suspension in 0.5 M NaCl, with 0.1 M sodium acetate, pH 5.0
Synonym: PBA-agarose

A4046 Matrix: acrylic beads
Activation: oxirane, with attachment through the amino group, with a 5-atom spacer
Ligand immobilized: 300-600 μmoles per gram
Form: lyophilized powder
Swelling: 1 g swells to approximately 4 mL

A8312 Matrix: 6% beaded agarose
Activation: epichlorohydrin, with attachment through amino group with a 9-atom spacer
Ligand immobilized: 40-90 μmoles per mL
Binding Capacity: 8-14 mg Peroxidase Type VI per mL
Form: suspension in water containing 0.002% chlorhexidine diacetate.

STORAGE/ STABILITY AS SUPPLIED:

All three products are stable at least two years stored at 2-8°C. Both A8312 and A8530 should be kept from freezing to avoid damage to the agarose bead structure.

GENERAL REMARKS:

Due to the attachment through the amino group, the effective group available to bind proteins is a phenylboronate group, which can form a temporary covalent bond with any molecule that contains a 1,2-cis-diol group. Porath offers a method of preparing the resin by epoxy-activation of beaded agarose.[2]

The phenylboronate ligand can be used directly with a molecule containing the cis-diol structure (to produce) a second ligand with more specific binding. Most nucleotides and nucleosides will bind to phenylboronate, so that they, in turn, bind other molecules. These resins can also be used to bind a variety of enzymes, for example, glucose-6-phosphate dehydrogenase and hexokinase, when $NADP^+$ is complexed with the column.[3] Lactamases have also been purified using PBA-agarose.[4] Serine proteases such as subtilisin, α-chymotrypsin and trypsin have been purified using aminoethyl phenylboronic acid to CH-Sepharose™.[5] Phenylboronic acid resins have been used for separation and quantitation of glycosylated hemoglobins.[6,7] In general, equilibration buffers should be of low ionic strength, with pH 7-9.[1]

Suggested protocol for binding nucleosides:[8]

Equilibrate a 1.5- to 2-mL column with 50 mM potassium phosphate containing 1.0 M NaCl, pH 7.8, for approx. 24 hours before use, using a flow rate of 2 mL/hour. Apply a mixture of nucleosides and deoxynucleosides (2.5 µmole of each) to the column in the same buffer (total volume 0.4 mL). Develop the column using the same buffer, collecting deoxynucleosides in the void volume; elute tightly-bound nucleosides with 100 mM sorbitol in buffer, collecting 1 to 2 mL fractions. Chromatography may be conducted at 4°C or at 25°C. Fractions may be monitored at 260 nm.

Gehrke et al. used 25 mM ammonium acetate buffer pH 8.8, with flow rate of 10-20 mL per hour (gravity flow) to wash the new resin, then 0.1 M formic acid, "which causes the resin to contract visibly", and again with 0.25 M ammonium acetate buffer pH 8.8. Samples were loaded in ammonium acetate buffer, and elution was done with 0.1 M formic acid.[9]

Suggested protocol for binding glycoproteins:[8]

For a column volume of 2 mL, apply protein 2-4 mg in approximately 250 µL of buffer: 50_mM_taurine/NaOH, pH 8.7, containing 20 mM $MgCl_2$ (more or less, depending on the glycoprotein).[8] Develop the column at a flow rate of 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.

REGENERATION:[1]

The resin can be washed using 10 column volumes of each:

1. 0.1 M borate buffer pH 9.8 containing 1.0 M NaCl;
2. 0.1 M borate buffer pH 9.8;
3. deionized water;
4. 2.0 M NaCl

The resin can be stored in 1 M NaCl (or the solution in which it was originally packaged) at 2-8°C with a bacteriostat. It should be equilibrate with starting buffer prior to use.[1]

REFERENCES:

1. Sigma Chromatography Kit Technical Bulletin; production information.
2. Porath, J., Methods in Enzymology, 34, 13-30 (1974). General methods and coupling procedures.
3. Bouriotis, V. et al., J. Chromatography, 210, 267-278 (1981).
4. Cartwright, S.J. and Waley, S.G., Biochemistry J., 221, 505-512 (1984).
5. Akparov, V.K. and Stepanov, V.M., J. Chromatography, 155, 329-336 (1978).
6. Mallia, A.K. et al., Analytical Letters, 14 (B8), 649-661 (1981).
7. Immobilized Affinity Ligand Techniques, Hermanson, G.T., *et al.*, Eds. (Academic Press, 1992), pp. 338, 339-392.
8. Affinity Chromatography: A Practical Approach, P.D.G Dean, W.S. Johnson and F. A. Middle, Eds., (IRL Press, 1985), p. 133.
9. Gehrke, C.W., Kuo, K.C., Davis, G.E. and Suits, R.D., J. Chromatography, 150, 455-476 (1978).

ADDITIONAL GENERAL REFERENCES:

Hjerten, S. And Li, J-P., J. Chromatography, 500, 543-53 (1990).
Renell, M. et al, Comp. Biochem. Physiol., B: Comp. Biochem., 81B(4), 819-822 (1985).
John, W.G. and Jones, A.E., Ann. Clin. Biochem., 22(1), 79-83 (1985).
Herold, David et al., Ann. Clin. Lab. Sci., 13(6), 482-488 (1983).
Yue, D.K. et al., Diabetes, 31(8), 701-705 (1982).