



Product Information

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 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

A4046 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

A8312 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.

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-SepharoseTM.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 μ mole 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 μ L of buffer: 50 mM taurine/NaOH, pH 8.7, containing 20 mM MgCl₂ (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:

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ADDITIONAL GENERAL REFERENCES:

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