



## Product Information

### p-AMINO BENZAMIDINE-AGAROSE

Product Number **A 7155**

Storage Temperature 2-8 °C

#### Product Description

Matrix: 4% beaded agarose, activated by cyanogen bromide

Spacer: 7-atom (Glycylglycine is attached via an amino group. EDAC condensation reaction on the free carboxyl group of the Gly-Gly with p-aminobenzamidine amino group results in final product).<sup>1</sup>

Binding capacity: 7-10 mg trypsin per mL resin

This product is tested for its binding capacity for trypsin, but the resin has been used in the purification of a variety of proteins including:  
Thrombin<sup>2,3</sup>

Adenylyl cyclase-activating protease from bovine sperm<sup>4</sup>  
Fibrinolytic enzyme from *Agkistrodon contortrix* contortrix<sup>5</sup>  
Trypsin from rat skin<sup>6</sup>  
Serine endopeptidase from rat mammary tissue<sup>7</sup>  
Glycosylation-enhancing factor (GEF)<sup>8-11</sup>  
Plasminogen activator from embryo lung culture<sup>12,13</sup>  
Plasminogen activator from human umbilical vein endothelial cells<sup>14</sup>  
Thrombin-like enzyme from *Bothrops atrox* venom<sup>15</sup>

#### Components

The agarose is a suspension in 0.5 M NaCl containing 0.02% thimerosal as preservative.

#### Preparation Instructions

The agarose beads should be well washed with an equilibration buffer to remove the thimerosal preservative. In the trypsin-binding assay, the equilibration buffer used was 50 mM Tris-HCl containing 0.5 M NaCl at pH 8.0. Trypsin is eluted using 10 mM HCl containing 0.5 M NaCl at pH 2.0.<sup>1</sup>

Thrombin was purified using as equilibration buffer 0.3 M imidazole-HCl at pH 7.35. The thrombin was eluted from the resin with this buffer with 0.2 M benzamidine added. Some plasmin also binds to the resin (since benzamidine has about the same  $K_i$  for thrombin and plasmin). High purity thrombin was obtained after a final ion-exchanged step on sulfo-ethyl Sephadex (SE-

Sephadex).<sup>2</sup> A closely related resin, p-chlorobenzamidine-agarose, was also used to purify thrombin, using 0.3 M phosphate buffer at pH 8.0, then eluted with 0.3 M phosphate buffer at pH 7.0 containing benzamidine.<sup>3</sup>

Binding may be generally temperature-dependent. Holleman and Weiss reported that the thrombin-like enzyme from snake venom was not bound to the resin at room temperature, only retarded in relation to the rest of the protein. However, at 4 °C, the enzyme was adsorbed and could be eluted specifically with benzamidine (0.15 M). Optimal equilibration buffer for loading and washing was 50 mM tris, with 0.4 M NaCl at pH 9.0.<sup>15</sup>

#### Regeneration

The resin should be given a series of washes with

- 10 column volumes (CV) 0.1 M borate buffer at pH 9.8 containing 0.5 M NaCl.
- 10 CV borate buffer without NaCl.
- 10 CV distilled or deionized water

Equilibrate with initial buffer for immediate re-use or store in 0.5 M NaCl with suitable bacteriostat at 2-8 °C.

#### Storage/Stability

The product should be stable at least two years stored at 2-8 °C. The resin should be protected from evaporation or freezing, since either will damage the bead structure.

#### References

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