

## Product Information

## p-Aminobenzamidine-Agarose

**A7155**

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 preservative.

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

The agarose beads should be well washed with an equilibration buffer to remove the 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

1. 10 column volumes (CV) 0.1 M borate buffer at pH 9.8 containing 0.5 M NaCl.
2. 10 CV borate buffer without NaCl.
3. 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.

## Reference

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