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).[1]

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[2,3]
- Adenylyl cyclase-activating protease from bovine sperm[4]
- Fibrinolytic enzyme from Agkistrodon contortrix contortrix[5]
- Tryptase from rat skin[6]
- Serine endopeptidase from rat mammary tissue[7]
- Glycosylation-enhancing factor (GEF)[8-11]
- Plasminogen activator from embryo lung culture[12,13]
- Plasminogen activator from human umbilical vein endothelial cells[14]
- Thrombin-like enzyme from Bothrops atrox venom[15]

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.[1]

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®).[2] 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.[3]

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 ºC, 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.[15]

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 ºC.

Storage/Stability

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

Reference

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