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Benzamidine **Sepharose** 6B

Affinity media

Instructions for Use

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71709600 AF

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1 Abstract

Benzamidine Sepharose™ 6B is p-aminobenzamidine covalently attached to Sepharose 6B by the epoxy coupling method.

p-Aminobenzamidine (PAB), is a synthetic inhibitor of trypsin-like serine protease. Trypsin and trypsin-like serine proteases bind to Benzamidine Sepharose 6B and can thus be used for purification and/ or removal of these substances. Trypsin, bovine thrombin, urokinase, human enterokinase, acrosin, native plasminogen, kallikrein, prekallikrein, collagenase and clostripain are some of the serine proteases that have been purified on Benzamidine Sepharose 6B.

For recombinant purification, Benzamidine Sepharose 6B can be used for removal of serine proteases such as thrombin and enterokinase after cleavage of purification tags.

Table 1. Medium characteristics.

Ligand density:	7 µmole p-aminobenzamidine/mL drained media
Available capacity[1]:	13 mg trypsin/mL drained media
Bead structure:	6% agarose
Bead size range:	45–165 µm
Mean particle size:	90 µm
Max linear flow rate[2]:	75 cm/h at 25°C, HR 16/10 column, 5 cm bed height
pH stability[3]	
Long term:	3–11
Short term:	2–13
Chemical stability:	Stable to all commonly used aqueous buffers
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.

1 The binding capacity was determinded in 50 mM Tris-HCl, pH 8.0 containing 0.5 M NaCl.

2 $$\text{Linear flow rate} = \frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$$

3 The ranges given are estimates based on our knowledge and experience. Please note the following: pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance. pH stability, short term refers to the pH interval for regeneration and cleaning.

2 Preparing the medium

Benzamidine Sepharose 6B is supplied pre-swollen in 20% ethanol. Prepare a slurry by decanting the ethanol solution and replacing it with binding buffer in a ratio of 75% settled media to 25% buffer before packing. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

3 Packing Sepharose 6B medium

Step	Action
1	Equilibrate all material to the temperature at which the chromatography will be performed.
2	De-gas the medium slurry.
3	Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4	Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5	Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6	Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see *Table 1, on page 4*, is typically employed during packing. ***Note:*** *If you have packed at the maximum linear flow rate, do not exceed 75% of this in subsequent chromatographic procedures.*

Step	Action
7	Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

For detailed description on column packing, refer to Handbook 18102229, which can be downloaded from *cytiva.com/protein-purification*

4 Using an adaptor

Adaptors should be fitted as follows:

Step	Action
1	After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2	Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3	Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the bump, and column and the sample application valve.

Step	Action
4	Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5	Lock the adaptor in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the medium bed is stable. Re-position the adaptor on the medium surface as necessary.

The column is now equilibrated and ready for use.

5 Binding of protein

A suitable binding buffer is 50 mM Tris-HCl, pH 8.0 containing 0.5 M NaCl. Good results are obtained at room temperature although the optimal temperature for binding is 4°C.

After the sample has been loaded, wash the medium with binding buffer until the baseline is stable.

6 Elution of protein

Bound substances can be eluted specifically or nonspecifically. To elute bound substances specifically, a competing agent such as p-aminobenzamidine can be used.

Competitive elution buffer:

20 mM p-aminobenzamidine in binding buffer.

Several methods may be used for non-specific elution of bound substances:

- A change in ionic strenght alters the degree of ionization of the charged groups at the binding site. Elution is normally complete at salt concentrations of 1 M or less of NaCl. Either step or continuous gradients may be used.
- A change in pH alters the degree of ionization of the charged groups at the binding. Either step or continuous gradients may be used.
- Reduction of the polarity of the elution buffer byaddition of dioxane (up to 10%) or ethylene glycol (up to 50%) may be used for elution of bound substances.
- Use of deforming agents like urea or guanidine hydrochloride is an alternative for elution of bound substances.

7 Regeneration

Depending of the nature of the sample, Benzamidine Sepharose 6B may be regenerated for re-use by washing the medium with 2–3 bed volumes of alternating high pH (0.1 M Tris-HCl + 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate + 0.5 M NaCl, pH 4.5) buffers. This cycle should be repeated 3 times followed by re-equilibration with 3–5 bed volumes of binding buffer.

If detergent or denaturing agents have been used during chromatography, these can also be used in the washing buffer.

8 Cleaning

In some applications, substances like denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the column with a detergent solution, e.g. 0.1% Triton X-100 at 37°C for one minute.

Re-equilibrate immediately with at least 5 bed volumes of binding buffer.

9 Storage

Benzamidine Sepharose 6B should be stored at 4°C to 8°C in the presence of a suitable bacteriostatic agent, e.g. 20% ethanol, at neutral pH.

10 Ordering Information

Product	Pack size	Code No.
Benzamidine Sepharose 6B	25 mL	17056801

11 References

1. Purification and characterization of a trypsin-like serine proteinase from rat brain slices that degrades laminin and type IV collagen and stimulates protease-activated receptor-2. *J. Neurochem. (2000), 74(4), 1731–1738*, Sawada, K. et al.
2. Purification of mast cell proteases from murine skin. *Exp. Dermatol. (1999), 8(5), 413–418*, Algermissen, B. et al.

3. Purification of rabbit liver aldehyde oxidase by affinity chromatography on benzamidine Sepharose 6B. *J. Chromatogr. (1989), 475 363-72, Stell, J. G. P. et al.*
4. The Crystal Structure of a T Cell Receptor in Complex with Peptide and MHC Class II. *Science 1999 December 3; 286: 1913–1921*. Reinherz, Ellis L. et al.

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71709600 AF V:6 07/2020