Benzamidine **Sepharose** 4 Fast Flow (low sub)

CUSTOM DESIGNED MEDIA

Benzamidine Sepharose™ 4 Fast Flow (low sub) is an affinity medium based on a highly cross-linked 4% agarose matrix, which enables rapid processing of large sample volumes. p-aminobenzamidine, a synthetic inhibitor of trypsin and trypsin-like serine proteases, is covalently bound to a long spacer arm attached to Sepharose 4 Fast Flow via a stable ether linkage (Fig 1). Benzamidine Sepharose 4 Fast Flow (low sub) has been used for purification of several enzymes such as trypsin and enterokinase (1, 2).

Key performance characteristics of Benzamidine Sepharose 4 Fast Flow (low sub) are:

- Ideal for industrial purification of serine proteases
- Selectivity similar to Benzamidine Sepharose 6B, but is more suitable for large-scale applications due to the Sepharose Fast Flow matrix
- Long spacer arm makes the small ligand easily available for binding target molecules

Characteristics

Table 1. Main characteristics of Benzamidine Sepharose 4 Fast Flow (low sub)

Matrix:	Macroporous, cross-linked 4% agarose
Average particle size:	90 µm
Ligand:	p-aminobenzamidine
Ligand density:	6–10 µmol/ml drained medium
Binding capacity:	Approx. 25 mg trypsin/ml drained medium
Flow velocity:	150–250 cm/h, 25 cm bed height, 0.1 MPa, distilled water in XK 50 column
pH stability*	
Long term:	2–8
Short term:	1–9

* For more information, see Stability below.

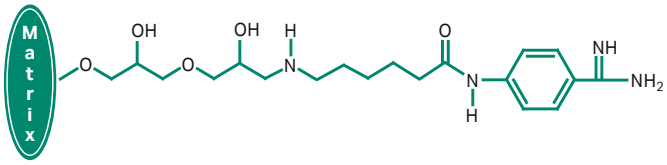

Fig 1. Partial structure of Benzamidine Sepharose 4 Fast Flow (low sub).

High sub versus low sub

Cytiva also offers Benzamidine Sepharose 4 Fast Flow (high sub), which is a medium designed for high capacity. It has a ligand density greater than 12 µmol/ml medium and a trypsin binding capacity greater than 35 mg/ml medium. For some applications, Benzamidine Sepharose 4 Fast Flow (high sub) induces strong interactions between ligand and target molecules, which may lead to reduced purity and recovery. Benzamidine Sepharose 4 Fast Flow (low sub) is designed to balance good capacity with high purity and recovery. The chemical construction is the same for both products without overlap in the ligand density specification. This makes it possible to evaluate the impact of ligand density in the purification process and select the optimal medium for specific purification needs.

Principles

Affinity chromatography exploits an immobilized ligand that adsorbs a specific molecule or group of molecules under suitable binding conditions and desorbs them under suitable elution conditions. Benzamidine Sepharose 4 Fast Flow (low sub) can be used for either positive affinity chromatography, such as isolation of a specific protease, or negative affinity chromatography, such as removal of proteolytic activity from a protein or peptide preparation. The binding and elution conditions depend on the target molecule, feed composition, and chromatography medium, and must be studied together with other chromatographic parameters (i.e., sample load, flow velocity, bed height, regeneration, cleaning-in-place, etc.) to establish the conditions that will bind the largest amount of target molecule, in the shortest time and with the highest product recovery.

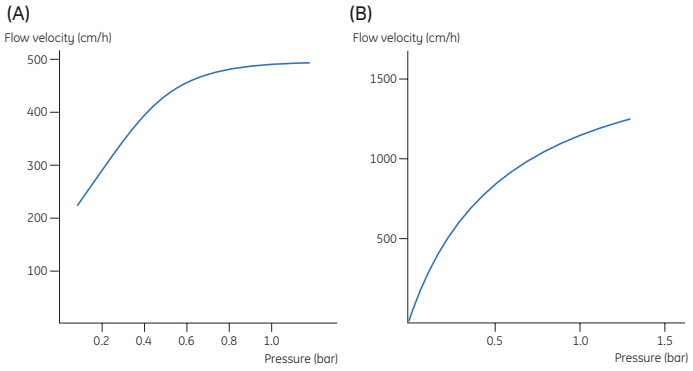

Fig 2. Pressure and flow velocity curve for Sepharose 4 Fast Flow in (A) K 50/30, bed height 15 cm, and (B) BP 113, bed height 5 cm.

We recommend a bed height of 10 to 15 cm to allow high flow rates to be used. As a guide, pressure and flow curves for the base matrix Sepharose 4 Fast Flow in a K 50/30 and a BP 113 column are shown in Figure 2. Trypsin purification gives an example of the typical binding and elution conditions that can be used; trypsin binds at 0.05 M Tris, 0.5 M NaCl, pH 7.4, and can be eluted with 0.5 M arginine, 0.05 M glycine, pH 3.0. The elution can be improved by adding competing agents such as arginine or aminobenzamidine.

Regeneration should restore the original function of the medium. Depending on the nature of the sample, regeneration is normally performed after each cycle followed by re-equilibration in start buffer. To prevent build up of contaminants over time, more rigorous protocols may have to be applied (see Cleaning-in-place and sanitization right).

Stability

The amide bond between the ligand and the spacer arm is stable over almost the entire pH range. However, at pH below 2 or above 8, the ligand is hydrolyzed to p-aminobenzamide and then to p-aminobenzoic acid without being detached from the matrix. Since only the benzamidine group has affinity for trypsin and other proteases, ligand hydrolysis will result in a decrease in trypsin binding capacity. This has been demonstrated in a study on Benzamidine Sepharose 4 Fast Flow (high sub), see Figure 3.

Benzamidine Sepharose 4 Fast Flow (low sub) has been stored successfully in 7.2 M guanidine hydrochloride, 0.1 M acetic acid, pH 3.0 for six weeks without a reduction in trypsin binding capacity, ligand density, or flow properties.

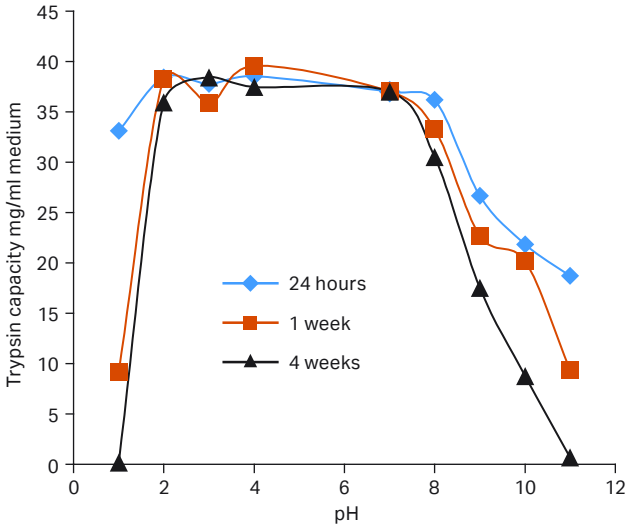

Fig 3. Benzamidine Sepharose 4 Fast Flow (high sub) has been tested for trypsin binding capacity after storage at pH 1–11 at ambient temperature for 24 hours, 1 week, and 4 weeks.

Cleaning-in-place and sanitization

A cleaning and sanitization protocol has to be designed for each application. Supportive information can be obtained from the regulatory support file.

Short term use of pH 1–9 can be used, since more than half of the trypsin binding capacity remains after 1 week (168 h) contact time at pH 9 (Fig 3). However, prolonged exposure to pH below 2 and above 8 should be avoided due to a slow decomposition of the matrix at low pH and hydrolysis of the ligand. A general recommendation is to use a guanidine hydrochloride solution to remove precipitated or denatured substances. For hydrophobically bound substances, a solution of nonionic detergent or ethanol is recommended.

For sanitization of Benzamidine Sepharose 4 Fast Flow (low sub), storage in 0.1 M acetic acid and 20% ethanol is recommended (3).

Storage

We recommend that the medium is stored in pH 4 buffer containing 20% ethanol at 4°C to 8°C. Benzamidine Sepharose 4 Fast Flow (low sub) is supplied pre-swollen in an acetate buffer, pH 4, containing 20% ethanol as a bacteriostat.

References

1. Kanamori, A. *et al*. Preparation of high-capacity affinity adsorbent using formyl carriers and their use for low- and high-performance liquid affinity chromatography of trypsin-family proteases. *J. Chromatogr*. **363**, 231–242 (1986).
2. Grant, D. *et al*. Optimization of conditions for the affinity chromatography of human enterokinase on immobilized p-aminobenzamidine. *Eur. J. Biochem*. **88**, 183–189 (1978).
3. Sofer, G. and Hagel, L. Cleaning, Sanitization, and Storage, in *Handbook of Process Chromatography*: A Guide to Optimization, Scale-up, and Validation, Academic Press, pp. 188–214 (1997).

Ordering information

Product	Quantity	**Code No.**
Benzamidine Sepharose 4 Fast Flow (low sub)*	100 ml	28-4108-01
	5 l	28-4108-03

Related Products

Product	**Quantity**	**Code No.**
Benzamidine Sepharose 4 Fast Flow (high sub)	100 ml	17-5123-01
	500 ml	17-5123-02
	5 l	17-5123-03
HiTrap™ Benzamidine FF (high sub)	2 × 1 ml	17-5143-02
	5 × 1 ml	17-5143-01
	1 × 5 ml	17-5144-01

* This product is part of our Custom Designed Media program. If you are interested in large-scale quantities, please contact your local Cytiva representative.

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