ECH-Lysine **Sepharose** 4 fast flow

CUSTOM DESIGNED MEDIA

ECH-Lysine Sepharose™ 4 Fast Flow is a group specific medium for isolation of plasminogen and plasminogen activator.

It is based on highly crosslinked 4% agarose thus enabling rapid processing of large sample volumes. L-Lysine is covalently bound to a long hydrophilic spacer arm attached to Sepharose 4 Fast Flow via a stable ether linkage, Figure 1.

ECH-Lysine Sepharose 4 Fast Flow was designed for industrial purification of plasminogen and plasminogen activator [1-5].

Characteristics

Mean particle size:	90 µm
Bead structure:	Macroporous crosslinked 4% agarose
Ligand density:	13–18 µmol Lysine/ml drained medium
Binding capacity:	> 1.5 mg Plasminogen/ml drained medium
Flow velocities:	min. 200 cm/h, 25 cm bed height, 0.1 MPa distilled water, XK 50 column

Recommended pH ranges

working range:	3–12
cleaning-in-place:	2–13

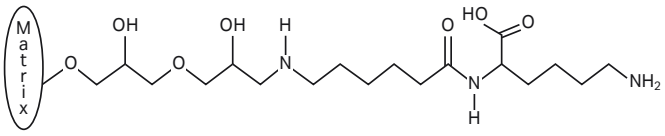

Fig 1. Partial structure of ECH-Lysine Sepharose 4 Fast Flow.

Principles

Affinity chromatography exploits an immobilized ligand that adsorbs a specific molecule or group of molecules under binding conditions and desorbs them under elution conditions.

These conditions depend on the target molecule, feed composition and the chromatography medium. For example, plasminogen adsorbs to ECH-Lysine Sepharose 4 Fast Flow when plasma (sodium chloride is added to a final concentration of 0.1 M) is pumped through the column. Unspecifically bound proteins are washed away with a buffer (50 mM sodium dihydrogen phosphate, 0.1 M sodium chloride pH 7.4). The plasminogen is then eluted with the desorption buffer (adsorption buffer + 50 mM e aminocaproic acid).

The binding conditions have to be studied together with the rest of the chromatographic conditions (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. We recommend a bed height of 10-15 cm to allow high flow rates to be used.

As a guide, pressure/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. Regeneration should restore the original function of the adsorbent. 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*).

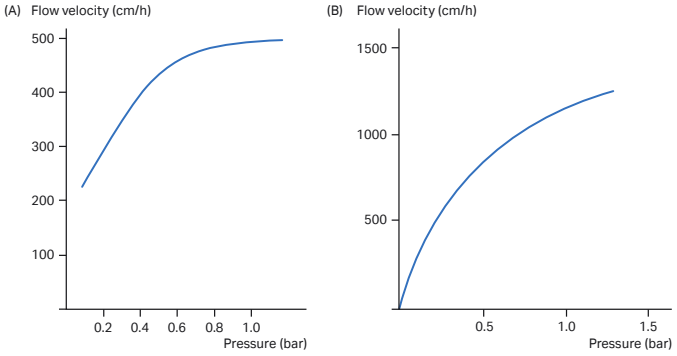

Fig 2. Pressure/flow velocity curve for Sepharose 4 Fast Flow in K 50/30, bed height 15 cm (A) and BioProcess™ 113, bed height 5 cm (B) (Work from Amersham Pharmacia Biotech).

This product is part of our Custom Designed Media program and is available for evaluation. It is not yet a standard product. If you are interested in large-scale quantities it is important to contact us as early as possible via your local Cytiva representative.

Stability

The amide bond between the lysine and the spacer arm is stable under alkaline conditions. The figure below shows the stability of ECH-Lysine Sepharose 4 Fast Flow after storage in 0.1 M and 1.0 M NaOH solutions at 20°C and 40°C for 400 days. The change in the Lysine content is insignificant after storage of 400 days at pH 13 (0.1 M, NaOH) at room temperature, see Figure 3.

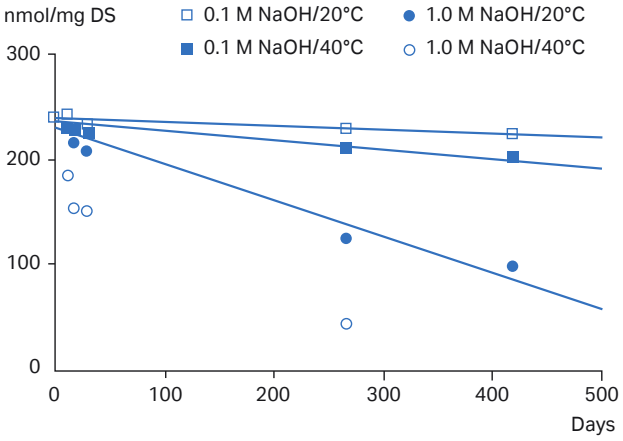

Fig 3. Ligand density (nmol Lysine/mg Dry Substance) after storage in 0.1 and 1 M Sodium hydroxide at 20°C and 40°C for 400 days.

Cleaning-in-place and sanitization

A cleaning or sanitization protocol has to be designed for each application. Generally sodium hydroxide (0.1–1 M) alone or in combination with sodium chloride (0.5–3 M) or ethanol (20–70%) is an effective sanitization agent. Prolonged exposure, i.e., several days, to pH greater than 13, see Figure 3, or lower than 2 should be avoided due to hydrolysis of the ligand at high pH and decomposition of the matrix at low pH. Strongly bound proteins can be removed with urea or guanidine hydrochloride [6].

Storage

We recommend that the medium should be stored at pH 5-7 in 20% ethanol. ECH-Lysine Sepharose 4 Fast Flow is supplied pre-swollen in a buffered 20% ethanol solution.

References

1. High-performance chromatographic method for the purification of tissue-type plasminogen activator. *J. Chromatogr.* **369**, 391-397, (1986). Matsuo, O. *et al.*
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3. Purification and characterization of tissue plasminogen activator kringle-2 domain expressed in *Escherichia coli. Biochemistry* **28,** 1884-1891, (1989). Cleary, S., Mulkerrin, M.G. and Kelley, R.F.
4. Large-scale purification of human tissue-type plasminogen activator using monoclonal antibodies. *Biochim. Biophys. Acta* **830,** 1-10, (1985). Einarsson, M., Brandt, J. and Kaplan, L.
5. Plasminogen: Purification from human plasma by affinity chromatography. *Science.* **170,** 1095-1096 (1970). Deutsch, D.G. and Mertz, E.T.
6. Cleaning, sanitization and storage, in Handbook of Process Chromatography: A Guide to optimization, scale-up, and validation. Academic Press, pp. 188-214 (1997). Sofer, G. and Hagel, L.

Ordering Information

Product	Pack size	Code No.
ECH-Lysine Sepharose 4 Fast Flow	500 ml	17-0902-02
ECH-Lysine Sepharose 4 Fast Flow	5 L	17-0902-04

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