GE Healthcare

Instructions 71-7095-00 AE **Affinity Media**

Lysine Sepharose 4B

Lysine Sepharose™ 4B is L-lysine immobilized to Sepharose 4B by the cyanogen bromide method. L-lysine is coupled via its α-amino group, leaving both the ε-amino and α-carboxyl groups free to interact with sample substances during chromatography.

It is designed for purification of molecules with biospecific or charge dependent affinity for L-lysine.

Lysine Sepharose 4B is a group specific adsorbent and has been used for isolation of plasminogen and plasminogen activator, separation of ribosomal RNA (rRNA) and purification of doublestranded DNA. The precise mechanism by which separation occurs is not fully understood but both electrostatic and stereospecific effects may contribute to the separation, depending upon the application.

Sepharose™

Table 1. Medium characteristics.

Ligand density:	4–7 µmol lysine/ml drained medium
Binding capacity:*	0.6 mg human plasminogen/ml drained medium 0.6–0.7 mg rRNA/ml drained medium
Bead structure:	4% agarose
Mean particle size:	90 µm
Bead size range:	45–165 µm
Max linear flow rate**:	75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH stability***	
Long term:	2–11
Short term:	2–11
Chemical stability:	Stable to all commonly used aqueous buffers
Physical stability:	Negligible volume variation due to changes in pH or ionic strength

* The binding capacity for human plasminogen was determined in 50 mM phosphate buffer, pH 7.5.

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

*** 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.

Contents

1. Preparing the medium

Lysine Sepharose 4B is supplied lyophilized in the presence of additives. These additives must be washed away at neutral pH.

Weigh out the required amount of lyophilized powder (1 g powder gives about 4 ml final medium volume) and suspend it in distilled water. The medium swells immediately and should now be washed for 15 minutes with distilled water on a sintered glass filter (porosity G3). Use approximately 200 ml distilled water per gram lyophilized powder, added in several aliquots.

Prepare a slurry with binding buffer in a ratio of 75% settled medium to 25% buffer. 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.

2. Packing Sepharose 4B

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 flow 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, is typically employed during packing.

Note: Do not exceed 75% of the packing flow rate in subsequent chromatographic procedures.

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

3. Using an adaptor

Adaptors should be fitted as follows:

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully add more buffer into the column to form an upward meniscus.
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 pump.
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 bed is stable. Repositioningthe adaptor on the medium surface as necessary.

The column is now equilibrated and ready for use.

4. Binding

Proteins which bind to Lysine Sepharose 4B do so around physiological pH. A recommended binding buffer is 50 mM phosphate buffer, pH 7.5.

For plasminogen purification the following procedure is recommended:

1. Equilibrate the medium with 2–3 bed volumes of binding buffer, 50 mM phosphate, pH 7.5.
2. Apply the sample. Up to 10 bed volumes of serum can be applied. After the sample has been loaded, wash the medium with binding buffer until the baseline is stable.
3. Add sodium chloride to the binding buffer to 0.5 M and elute loosely or non-specifically bound substances.
4. Elute plasminogen with 0.2 M ε-aminocaproic acid in distilled water.
5. Re-equilibrate the medium with at least 3 bed volumes of binding buffer. It is recommended to wash the medium with 0.2 M ε-aminocaproic acid in 50 mM phosphate buffer, pH 7.5, containing 1 M NaCl every fifth cycle.

   The ε-aminocaproic acid may be removed from the plasminogen by desalting on Sephadex™ G-25.

5. Elution

Lysine Sepharose 4B is a group specific adsorbent with affinity for a variety of biomolecules. Some proteins interact biospecifically due to their structural similarity with the ligand while others bind in a less specific manner by electrostatic interactions.

- Specifically bound biomolecules, like plasminogen, may be eluted by competitive elution. Use of a competing agent for either the ligand or the target molecule, e.g. ε-aminocaproic acid in the buffer will elute specifically bound substances. Plasminogen normally elutes with 0.2 M ε-aminocaproic acid. Either step or continuous gradients may be used.
- Less specifically bound biomolecules can be eluted with increased ionic strength. Elution is normally complete at salt concentrations of 2 M or less of NaCl. Either step or continuous gradients may be used.
- Elution can also be achieved using temperature gradients. The affinity of e.g. rRNA to Lysine Sepharose 4B is affected by temperature. As the temperature is reduced a higher concentration of salt is required to elute each RNA species.

6. Regeneration

Depending on the nature of the sample, Lysine Sepharose 4B 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 at least 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.

After purification of plasminogen, the medium should be regenerated by washing with several bed volumes of 50 mM phospate buffer, pH 7.5, containing 1 M NaCl and 0.2 M ε-aminocaproic acid.

After purification of nucleic acids, wash the medium with at least 5 bed volumes of 50 mM phospate buffer, pH 7.5, containing 2 M NaCl.

7. 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.

8. Storage

Lyophilized Lysine Sepharose 4B should be kept dry and stored below 8 °C.

Swollen medium should be stored at 4–8 °C in presence of a bacteriostat, e.g. 20% ethanol. The medium must not be frozen.

9. Further Information

Check www.gehealthcare.com/protein-purification for more information. Useful information is also available in the Affinity Chromatography Handbook, see ordering information.

10. Ordering Information

Product	Pack size	Code No.
Lysine Sepharose 4B	15 g	17-0690-01
Lysine Sepharose 4B	250 g	17-0690-09
HiTrap™ Desalting Column	5 x 5 ml	17-1408-01
HiPrep™ 26/10 Desalting Column	1	17-5087-01
PD-10 Desalting Columns	30	17-0851-01
Literature		
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

www.gehealthcare.com/protein-purification
www.gehealthcare.com

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