Instructions for Use

Lysine HyperD®

Affinity Chromatography Resin

1 Product Description

Lysine HyperD® resin is a high speed high capacity affinity preparative resin for the purification of biological molecules that bind to lysine, such as plasminogen from human or animal species plasma. The resin provides high binding capacity at high flow rates.

Lysine HyperD® affinity resin employs a high-capacity hydrogel polymerized within the large pores of a rigid bead. This design combines the desirable characteristics of a soft, high capacity hydrogel with the high dimensional stability of a rigid bead.

Lysine HyperD® resin is manufactured at ISO 9001:2008 and ISO 14001:2004 compliant manufacturing facility.

Lysine HyperD® resin is used for the purification of therapeutic proteins that bind to lysin in regulatory-approved production processes in columns, as well as for processes in development producing material for preclinical and clinical trials, in columns > 100 litres.

It is supplied in a variety of package sizes in 1 M NaCl containing 20% ethanol.

2 Properties

Particle size	70 µm (av.)
Ligand	L-lysine
Working pH	3 – 13
Volume changes due to pH and ionic strength	Non compressible
Pressure resistance	70 barg (1,000 psig)
Working pressure	< 3 barg (44 psig)

2.1 Main Benefits

- Very high productivity as a consequence of a high binding capacity and the ability to run at high flow rates.
- Rapid packing due to the high density of lysine resin which settles in a few minutes.
- HyperD® resin is very rigid and allows the use of high flow rates without pressure increase or shrinking or swelling of the resin.
- Minimized leakage due to the stable chemical link of the lysine molecule to the resin. Lysine HyperD® can be occasionally washed with 0.1 M sodium hydroxide for cleaning.

2.2 Applications

Lysine HyperD® is used for the purification of plasminogen from human or animal species plasma. It can be used to prepare for example plasmine-free serum for cell culture, by removal of the plasminogen present in the whole serum. Plasminogen contains different subclasses that can be discriminated by gradient elution using ε-amino caproic acid.

Lysine HyperD® has been developed to improve the productivity of process-scale molecules such as plasminogen or plasminogen activators, in allowing a higher binding capacity at higher flow rates than conventional agarose-based resins.

In Lysine HyperD®, the L-lysine moiety is immobilized covalently by mostly the α-amino groups, via an aminoalkyl linkage. The general recommendations of use for Lysine HyperD® are very similar to those of conventional lysine-immobilized resins.

3 Column Packing

3.1 Packing a Small column (≤ 5cm I.D.)

Lysine HyperD® is compatible with traditional low or medium pressure chromatography columns and equipment.
For preliminary laboratory studies, a column equipped with an adjustable piston, of 1 cm I.D. x 20 cm length, facilitates optimal packing.
Direct scale-up is accomplished by maintaining bed height constant while diameter is increased. We recommend a chromatography system able to deliver a minimum linear flow rate of 300 cm/h for packing. 600 cm/h will be recommended when a pressure versus flow rate curve is required.

3.1.1 Preparing the Resin Slurry

⚠ **Do not use stainless steel paddles or magnetic stirrers as they may damage the beads and create fine particles.**

1. Gently agitate the container to fully suspend the resin.
2. Depending on the desired bed volume, transfer a suitable volume of slurry to a graduated beaker including a "practical" excess of resin (20 to 25% of the desired bed volume are recommended).
3. Allow the resin to settle and remove the supernatant. Add 3 to 5 volumes of packing buffer. (i.e., 50 mM Sodium Phosphate pH 8.0) be used.
4. Gently agitate the slurry and let the resin settle. Remove the supernatant and add 3 to 5 volumes of fresh packing buffer.
5. Repeat step 4, three times minimum in total.
6. Gently agitate the slurry, pour it into a measuring cylinder and allow the resin to settle.
7. Remove the supernatant and add a volume of packing buffer equal to one-half the volume of settled resin. A slurry of 67% (v/v) — the concentration recommended for packing — is obtained.

Alternatively, the slurry concentration can be estimated by centrifuging between 20-g and 200-g about 10 mL of homogenized slurry in a graduated vial tube for 5 minutes. The concentration of slurry expressed as:
[resin volume | total volume (resin + supernatant) x 100] can be directly estimated in the tube. The 10 mL slurry can be poured back into the total suspension before packing.

3.1.2 Preparing Column and System for Packing

1. Prime the solvent delivery system, lines and valves to assure that all air is displaced. Assure that top and bottom frits or nets are fully wetted and free of air. Fill the column with packing buffer and operate the system over a range of flow rates representative of values that will be used during packing and anticipated chromatographic procedures.
   Record pressure associated with the empty column and system (including detectors, etc...).
2. Determine pressure | flow characteristics for the empty system, taking into account recommendations concerning flow rate during packing and chromatography operation.
3. Stop the pump, close the column outlet, and remove the upper flow adaptor or piston.
4. Open the outlet and drain buffer from the column, leaving 1 – 4 cm of buffer above the bottom frit.
   Following completion of blank pressure | flow measurements, proceed with column packing as described below.

3.1.3 Packing the Column

1. Gently resuspend the slurry and pour it into the column in one continuous motion against the wall of the glass tube to minimize introduction of air bubbles (if the pouring process is done in several motions, gently homogenize the slurry in the column using a plastic rod).
2. Fill with packing buffer (i.e., 50 mM Sodium phosphate, pH 8.0) up to the top of the glass tube. Allow the suspension to settle so that a layer of clear supernatant ≤ 1 cm is visible at the top of the column.
3. Connect the upper piston to the system and prime with packing buffer to ensure that no air is trapped under the net or frit. Stop the pump and insert the adjustable piston into the column. Tighten it.
4. Open the column outlet and operate the pump at a selected linear velocity of 300 cm/h (~3.9 mL/min for a 1 cm I.D. column) for efficient packing.
5. When the top of the bed stabilizes, stop the pump and untighten the piston. Position the adjustable piston at the top of the packed resin, leaving no visible space between the frit and packed resin at any point around the circumference.
6. Operate the pump again and repeat the adjustment of the piston until no visible space appears under flow (300 cm/h) between the frit and the top bed.

4 Working Protocol and Basic Conditions for the Purification of Plasminogen From Human Plasm

4.1 Sample

Plasma sample diluted with an equal volume of column equilibration buffer.

4.2 Experimental Procedure

— Equilibrate the column in a 50 mM phosphate buffer, pH 8.
— Load sample equilibrated in buffer above.
— Wash with 50 mM phosphate buffer + 0.3 to 0.5 M NaCl to elute loosely bound contaminants (such as albumin).
— Elute the plasminogen with a gradient of 0.2 to 0.5 M ε-amino caproic acid in a 50 mM phosphate buffer, pH 8.
— Regenerate with 0.1 M NaOH, 2 column volumes during 30 min.
— Wash with 50 mM phosphate buffer + 0.3 to 0.5 M NaCl, 1 – 2 column volumes.
— Equilibrate in starting buffer.

We recommend to operate at 300 cm/h for maximum productivity.

5 Regeneration and Cleaning

In order to avoid frequent regeneration, it is advisable to introduce into the column only samples and buffers that are clear and previously filtered to 0.2 µm. Be sure that changes in pH and ionic strength during elution do not cause precipitation of sample components.

Nevertheless, after repeated use and if necessary, Lysine HyperD® resin may be regenerated in column or in batch.

The following suggestions may be checked first for their degree of efficiency:

Situation	Recommendation
General Cleaning-In-Place (CIP)	Wash extensively with 3 M NaCl as a first trial.
Hydrophobic Contaminants	Wash with 8 M urea, or 6 M guanidine hydrochloride or an aqueous solution of non ionic detergent (1% Triton™ X-100*), 1 – 2 cv.
Unknown impurities	Wash with 0.1 M sodium hydroxide solution. 1 – 5 cv of this solution are generally sufficient to remove alkaline soluble material. After treatment, neutralize the column immediately by washing with strong buffer solution. Flow rate should be between 0.5 and 3 column volumes/h.

After packing or between runs, it can be necessary to remove pyrogens from the resin. The above suggested sodium hydroxide solutions are effective to remove pyrogens and in most cases to inactivate microorganisms.
After treatment, the column must be reequilibrated with the normal sterile pyrogen-free buffer.

6 Thermal Stability and Storage

Temperature of use	2 – 30 °C (36 – 86 °F)
Storage temperature	2 – 30 °C (36 – 86 °F) (2 – 8 °C | 36 – 46 °F once opened)
Recommended storage solution (between runs)	Neutral buffer containing bacteriostatic agents such as 1 M NaCl and 20% (v/v) ethanol.
⚠	Product must never be frozen
i	Product is shipped at ambient temperature

7 Ordering Information

Pack Size	Part Number
25 mL	20059-036
100 mL	20059-028
1 L	20059-010
10 L	20059-044

For more information, please contact our technical service.

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Sartorius Stedim Chromatography Resins SAS
48 avenue des Genottes
F-95800 Cergy Saint Christophe
www.sartorius.com

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