Instructions for Use

Heparin HyperD® M

Affinity Chromatography Resin

1 Product Description

Heparin HyperD® M resin is a high speed high capacity affinity preparative resin for the purification of biological molecules that bind to heparin, such as coagulation factors, growth factors, lipoproteins, etc... The resin provides high binding capacity at high flow rates Heparin HyperD® M 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. Heparin HyperD® M resin is manufactured at ISO 9001:2008 and ISO 14001:2004 compliant manufacturing facility.

Heparin HyperD® M resin is used for the purification of therapeutic proteins that bind to heparin 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 1 M NaCl containing 20% ethanol.

2 Properties

Particle size	80 µm (av.)
Dynamic sorption capacity for human ATIII (10% breakthrough)[1]	25 mg/mL
Ligand	Porcine heparin
Immobilized Heparin/mL of resin	5 – 10 mg/mL
Recommended operating pH range	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)

[1] Determined using hu ATIII at 72.5 UI/mL in 20 mM Tris-HCl, 0.3 M NaCl, pH 7.4. Elution with 20 mM Tris-HCl, 2 M NaCl, pH 7.4 at a flow rate of 600 cm/h, 10 cm (4 in.) bed height.

2.1 Main Benefits

- Very high productivity in making Antithrombin III as a consequence of a high binding capacity and the ability to run at high flow rates.
- Rapid packing due to its high density, it 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.
- Leakage is minimized due to the stable chemical link of the heparin molecule to the resin. Heparin HyperD® M withstands 0.1 M sodium hydroxide for cleaning.

2.2 Applications

There are many fields of applications of Heparin HyperD® M resin which are related to the different types of interactions of native heparin.These interactions may be specific as with certain coagulation factors, or may be due to a more complex ionic interaction.

Seven major groups of proteins can be purified on Heparin HyperD® M:
— Coagulation factors such as ATIII, Factor IX, Factor VII, Factor XI, Factor XII and XIIa.
— Lipoprotein lipases are enzymes which participate in lipid metabolism. Forming ionic complexes with heparin, immobilized heparin provides a suitable means for their purification. There are numerous reports on the purification of lipoprotein lipases from serum, mammalian heart, adipose tissue and bovine milk.
— Lipoproteins (LDL, VLDL, VLDL apoprotein, HDL) may form an insoluble complex with heparin in the presence of divalent cations.This property is exploited in the separation of serum lipoproteins on immobilized heparin (e.g. lipoprotein elimination from serum to reduce interference with enzymatic assays).
— Growth hormones.
— Growth factors: FGF, ECGF.
— DNA- and RNA-related enzymes as heparin is an inhibitor of DNA- and RNA-polymerases and interacts with numerous DNA- and RNA-dependent enzymes. These properties are used to purify a wide variety of enzymes (e.g. polymerases, restriction endonucleases).
— Other applications: immobilized heparin has been used for the purification of various other enzymes (e.g. collagenase, ∝-L-iduronidase, hyaluronidase and lysozyme), fibronectin, fibronectin fragments and hormones receptors.

2.3 Stability

2.3.1 Temperature Stability
As a result of the nature of the matrix and that of the chemical link, Heparin HyperD® M resin is autoclavable at 121 °C (250 °F) for 20 min.

2.3.2 Chemical Stability
The pH stability is the same as the free soluble heparin: between 3 and 13. Dissociating agents and detergents have generally no effect on heparin resin. Treatments of Heparin HyperD® M resin with 8 M urea, 6 M guanidine hydrochloride and 1% Triton™ X-100* led to no change when tested with bovine ATIII.

3 Column Packing

3.1 Packing a Small Column (<= 5cm I.D.)
Heparin 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., 20 – 50 mM Tris-HCl, pH 4 – 9.0) 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 flowadaptor 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 homo genize the slurry in the column using a plastic rod).
2. Fill with packing buffer (i.e., 20 – 50 mM Tris-HCl, pH 7.4 – 9.0) 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 Conditions and Basic Protocol for ATIII Purification

4.1 Sample
The sample is equilibrated in 20 mM Tris-HCl, 300mM NaCl, pH 7.4.

4.2 Experimental Procedure
- Equilibrate Heparin HyperD® M in 20 mM Tris-HCl, 300 mM NaCl, pH 7.4 with about 5 column volumes.
- Load the sample and wash the column with approx. 10 column volumes of equilibration buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.4).
- Eluate ATIII with 20 mM Tris HCl, 2 M NaCl, pH 7.4.
- When necessary, wash the column with 3 M NaCl.

5 Other Recommendations
For the purification of certain proteins, buffers should contain Ca++ and Mg++ ions.

Elutions may be performed with NaCl gradients, dissociating agents and | or detergents (DOC, Triton™ X-100*, SDS).

We recommend operation at 300 cm/h for maximum productivity.

6 Regeneration and Cleaning
In order to avoid frequent regeneration, only use samples and buffers that are clear and previously filtered to 0.2 µm. Make sure that changes in pH and ionic strength during elution do not cause precipitation of sample components.

If necessary, Heparin HyperD® M 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.
Hydrophobic Contaminants	Wash with with 8 M urea, 6 M guanidine hydrochloride and 1% Triton™ X-100*.
Alkaline Soluble Contaminants	Wash with 0.1 M sodium hydroxide solution. 1 – 5 cv. After treatment, neutralize the column immediately by washing with strong buffer solution. Flow rate should be between 0.5 – 3 cv/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 sterile, pyrogen-free running buffer or storage solution.

7 Thermal Stability and Storage

Temperature of use	2 – 30 °C
Storage temperature	2 – 8 °C
Recommended storage solution (between runs)	Neutral buffer containing 1 M NaCl and 20% (v/v) ethanol
⚠	Product must never be frozen. Avoid long exposure to light.
i	Product is shipped at ambient temperature

8 Ordering Information

Pack Size	Part Number
25 mL	20029-039
100 mL	20029-021
1 L	20029-013
10 L	20029-054

For more information, please contact our technical service.

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