BIOSEPRA® Heparin HyperD® M

Heparin HyperD® M
Affinity Chromatography Sorbent

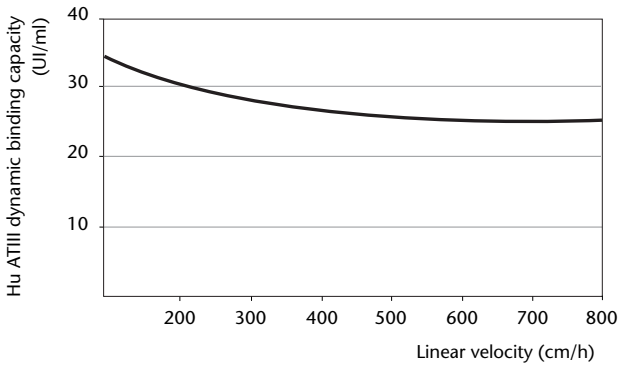
BioSepra® Heparin HyperD® M composite chromatography sorbent is the most technologically advanced high speed, high capacity affinity preparative sorbents for the purification of biological molecules that bind to heparin, such as coagulation factors, growth factors, lipoproteins... The sorbent provides high binding capacity at high flow rates.

Heparin HyperD® M unique composite structure was chosen to provide superior dynamic capacity at high linear velocities.

HyperD® sorbent is comprised of a porous rigid mineral bead containing heparin bound hydrogel filled pores. BioSepra holds U.S. patent 5,268,097 (1993) covering the unique properties of HyperD® sorbents and U.S. patent 5,234,991 (1987) on other "supported gel" or "gel-in-a-shell" sorbents.

Heparin HyperD® M has an average particle size of 80 µm and is used for preparative scale purification of ATIII. The sorbent can be packed in column sizes from ml to more than hundred liters and operated at high flow rates with low backpressure.

Figure 1 : Dynamic binding capacity vs. linear velocity.



Column dimensions: 0.46 cm I.D. x 10 cm; Sample: hu ATIII at 72.5 UI/ml; Equilibration buffer: 20 mM Tris-HCl containing 0.3 M NaCl, pH 7.4; Elution buffer: 20 mM Tris-HCl containing 2 M NaCl, pH 7.4.

Table 1. Heparin HyperD® M Main Properties.

Particle size	80 µm (av.)
Dynamic binding capacity for hu ATIII (600 cm/h)	> 25 mg/ml*
Ligand	Porcine heparin
Recommended operating pH range	3-13
Volume changes due to pH and ionic strength	Non compressible
Pressure resistance	70 bar (1,000 psi)

* Capacity 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 bed height.

The main benefits of Heparin HyperD® M sorbent are:

- Rapid packing due to the high density of heparin sorbent which settles in a few minutes.
- HyperD® sorbent is very rigid and allows the use of high flow rates without pressure increase or shrinking or swelling of the sorbent.
- Heparin leakage is minimized due to the stable chemical link of the heparin molecule to the sorbent.

Heparin HyperD® M is available as ready-to-use labpacks suspended in 1 M sodium chloride with 20 % ethanol as bacteriostatic. Larger bulk quantities are also available upon request.

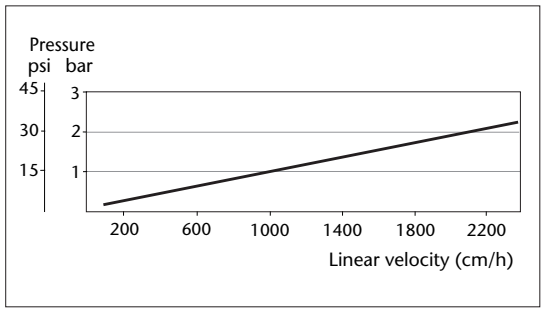
Capacity.

Heparin HyperD® M maintains high binding capacity, even at extremely high linear velocity. It is commonly used at large scale for the production of pharmaceutical grade ATIII. Production scale columns (>100 L) can be operated at high linear velocities (>200 cm/h) while maintaining capacity with minimal backpressure (Figure 2). Its capacity is higher than 25 UI/ml even at 600 cm/h with a 10 cm bed height (Figure 1).

Stability.

The non compressible HyperD® matrix can withstand very high flow rates without any risk of bed collapse. As a result, Heparin HyperD® M can be used with LPLC, MPLC or HPLC systems. An HPLC purification can be performed in less than 10 minutes using a 0.46 I.D. x 5 cm column. Faster purification saves user time and preserves the biological integrity of the purified proteins. The mechanical properties of Heparin HyperD® M sorbent remain constant across a wide range of velocities. Minimum pressure drop,

Figure 2: Pressure vs. linear flow velocity.



Column: 0.46 cm I.D. x 10 cm; Buffer: 20 mM Tris-HCl containing 0.3 M NaCl, pH 7.4.

even at high linear velocity, assures direct, predictable scale up to any volume (see Figure 2).

Mechanical and chemical stability.

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

Heparin HyperD® M can be cleaned with sodium hydroxide in concentrations of 0.01 to 0.1 M.

Validation.

The heparin used for the production of Heparin HyperD® M has a North American origin and is from porcine intestinal mucosa. The heparin is produced in compliance with the applicable requirements of the FDA's Good Laboratory Practices and Good Manufacturing Practices regulations.

A validation file can be provided to industrial customers to support the regulatory requirements for producing clinical and approved therapeutics.

Applications.

Heparin is a mucopolysaccharide known for its anticoagulant and clarifying actions.

Heparin is essentially composed of equimolar quantities of glucosamine and glucuronic acid, alternatively linked by α-1,4 glycosic bonds.

A certain number of its hydroxyl groups are esterified with sulfuric acid, especially those on C-6 of glucosamine. Other groups are also sulfated, including C-3 of glucosamine and C-2 of glucuronic acid. The main characteristic of heparin is that it contains a large number of amino groups combined with sulfate groups, the latter being quite labile in acidic medium.

The molecule contains small quantities of other sugar, such as galactose and xylose, and amino acids, e.g. serine, which explains positive ninhydrin reactions.

As a result of its composition and its biochemical role, heparin has the property to combine with a number of proteins, enzymes and in general with polycationic organic compounds. It is also combined with alkaloids, antibiotics, stains and hormones.

There are many fields of applications of Heparin HyperD® M sorbent 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 (polymerases, restriction endonucleases,...).
- Other applications: immobilized heparin has been used for the purification of various other enzymes (collagenase, α-L-iduronidase, hyaluronidase and lysozyme), fibronectin, fibronectin fragments and hormones receptors.

Ordering Information

Product	Cat. No.	Size
Heparin HyperD® M	20029-039	25 ml
	20029-021	100 ml
	20029-013	1 L
	20029-054	10 L

References

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LPN BT200080 - 12/2004