HyperTrap Heparin HP Column

Product description

HyperChrom Heparin HP Agarose is an affinity chromatography medium made by covalently coupling heparin to an agarose base. Heparin is a naturally occurring glycosaminoglycan that has affinity with a variety of biomolecules and can also be used as an ion exchange ligand. This chromatography medium can be used to isolate and purify coagulation factors, antithrombin III, growth factors, interferon, lipoprotein lipase, and enzymes for nucleic acid and steroid receptors. Compared to HyperChrom Heparin FF Agarose, the HyperChrom Heparin HP Agarose has a finer particle size and provides higher resolution.

HyperTrap Heparin HP Column uses HyperChrom Heparin HP Agarose as a chromatography medium for the isolation and purification of coagulation factors, antithrombin III, growth factors, interferon, lipoprotein lipase, and enzymes for nucleic acid and steroid receptors.

The main body and inner plug of the pre-installed column tube are made of PP, and the inside and outside are polished, and the sieve plate is made of HDPE material, which has excellent physical properties and chemical resistance, corrosion resistance, anti-aging, long life and good safety. 1) Ready-to-use, convenient and fast. 2) Can be used with syringes, peristaltic pumps or chromatography systems. 3) It can be used in series to increase the sample processing capacity.

Product parameters

1. HyperTrap Heparin HP Column preloaded column parameters

Pre-assembled column specification/name	1 mL	5 mL
Dimensions (IDxH)	7.7 x 25 mm	16 x 25 mm
Withstand pressure	0.3 MPa	0.3 MPa
Workflow Speed*	1 mL/min	1-3 mL/min
Operating temperature	4 - 30°C	4 - 30°C
Storage condition	Store the components at 4°C for 5 years.	

*This flow rate is the recommended common flow rate, the pressure resistance, and the use of different types of chromatography packing in the pre-assembled column will be different, please refer to the product manual of the corresponding chromatographic filler.

2. HyperTrap Heparin HP Column chromatography media parameters

Dame	Description
Chromatography media type	Heparin affinity chromatography mediator
Ligation	heparin
Scaffolding	Highly cross-linked agarose
Average particle size	34 μm
Ligand density	~10 mg/mL chromatography medium
Flow rates are recommended	90-150 cm/h
Maximum flow rate	200 cm/h
Withstand pressure	0.3 MPa
Use temperature	4-30℃
pH stability *	4-12
Chemical resistance	Common aqueous solution, 4 M NaCl, 0.1 M NaOH, 0.05 M sodium acetate (pH4), 6 M guanidine hydrochloride, 8 M urea, 70% ethanol**
* After 7 days of storage of chromatography medium at 40 °C and pH 4-12, its physicochemical properties and functions did not change significantly. **70% is v/v, volume ratio.	

Experimental manipulation

1. Preparation of buffers

Buffer type	Buffer components
Balance/Bind/Wash Buffer	0.02-0.05 M PB or Tris, pH 7.0-8.0, 0.15 M NaCl can be added to reduce non-specific adsorption.
Elution Buffer	0.02-0.05 M PB或Tris, 1-2 M NaCl,pH 7.0-8.0

2. Sample preparation

Prepare samples for purification.

3. Chromatographic conditions

- Flow rate selection: Linear flow rate of 90-150 cm/h is generally selected according to the height of the column bed.
- Sample preparation: To prevent the sample from clogging the column, the sample needs to be filtered with a 0.2/0.45 μm (after inclusion body disruption) microporous membrane before loading, and it is recommended that the pH and conductivity of the sample be adjusted to be consistent with the equilibrium buffer (the pH and conductivity of the sample can be adjusted by dilution, ultrafiltration, and desalting）。

4. Preloaded column setup

(1) Open the package and remove the preloaded column.

(2) Connect the pre-packed column: unscrew the upper and lower plugs of the pre-packed column and connect it to the chromatography system (peristaltic pump or syringe, etc.), rinse the pre-packed column with pure water 2-3 times the column volume to drain the preservation solution (generally 20% ethanol). In order to prevent air bubbles from entering, pure water can be dripped and filled at the inlet end of the pre-loaded column before connecting the system connector.

(3) Cleaning and disinfection: For the first use, it is recommended to clean and disinfect the column and rinse 2 times the column volume with pure water or buffer. For recommended cleaning and disinfection buffers, refer to subsequent cleaning and regeneration procedures.

(4) Sample preparation: To prevent the sample from clogging the column, it is recommended that the sample be filtered with a 0.45 μm microporous membrane before loading.

5. **Column efficiency determination** (optional).

Select one of the two test methods shown in the table below for column effectiveness testing. Use the mobile phase equilibrium chromatography column to the baseline to be stable, load the sample into the chromatography column, continue to use the mobile phase for rinsing, and after the chromatographic peak is completed to return to the baseline, end the run, integrate the chromatographic peak, and evaluate the loading effect.

Table 2 Statistical table of two column efficiency measurement methods

	Acetone method	NaCl method
sample	1% (v/v) acetone in water	2 M NaCl in water
Sample volume	1% column volume	1% column volume
Mobile phase	water	0.2 M NaCl in water
velocity of flow	30 cm/h	30 cm/h
Detector	UV 280 nm	electrical conductivity

The main evaluation criteria for the effect of column loading are N/m (number of plates per meter) and As (symmetry factor), which are calculated as follows:

$$\frac{N}{m} = 5.54x(\frac{V_R}{W_h})^2 \times \frac{1}{L}$$

$$As = {}^{b}/_{a}$$

Column efficiency qualification standards: N/m > 3000, 0.8 < As < 1.5

*Parameter Notes:

L = column height, VR = reserved volume, W_h = half-peak width, a = left half-peak width at 10% peak height, b = right half-peak width at 10% peak height

6. **Chromatographic steps**

(1) Equilibrium: Use Balance/Bind/Wash Buffer to fully equilibrate the column to pH and conductivity stable and substantially consistent with the equilibration buffer, which typically requires 3-5 times the column volume.

(2) Sample loading: Determine the sample loading volume and amount on the Hyper Chrom Heparin HP Agarose based on the binding load measured in the pilot experiment.

(3) Washing: Rinse the column with Balance/Bind/Wash Buffer or other suitable buffers until UV stable and return to baseline.

(4) Elution: Elution is achieved by increasing the concentration of salt ions, which can be gradually increased by linear gradient or step gradient to elute molecules with different binding strengths.

(5) Regeneration: Rinse the column with buffer containing high salt such as 2 M NaCl.

(6) Rebalancing: Re-equilibrate the chromatography column with Balance/Bind/Wash Buffer.

7. Cleaning and recycling

As the number of uses of the chromatography medium increases, contaminants (e.g., lipids, endotoxins, proteins, etc.) accumulate on the chromatography column. Regular in-place cleaning is essential to keep the column in stable working condition. Determine the frequency of in-place cleaning according to the degree of contamination of the chromatography medium (if the contamination is serious, it is recommended that in-place cleaning should be carried out after each use to ensure repeatable results and extend the working life of the chromatography medium).

For different types of impurities and contaminants, cleaning can be carried out under the following conditions:

- Removal of strongly binding proteins: Wash with 2 M NaCl solution in 5x column volume.
- Denatured and removal of precipitated proteins: first wash with 0.1 M NaOH solution in 5x column volume, then wash the lye with 5-10 column volumes of purified water. It can also be washed with 6 M guanidine hydrochloride or 8 M urea.
- Removal of hydrophobic and lipid substances: 0.1-0.5% nonionic detergent washed followed by rinse with 5-10 column volumes of purified water.

  Note: The flow rate can be selected from 30-60 cm/h during the cleaning process; When the blockage is serious, reverse cleaning can be used.

8. sterilization

In order to reduce the microbial load, it is recommended to use 0.5~1 M NaOH solution to treat the chromatography medium with a processing time of 15~30 min.

9. stockpile

Unopened chromatography media, please keep in the original container; The completed chromatography column is first soaked with 20% ethanol solution and then the upper and lower column heads are closed. The storage environment is 4~30°C.

10. **Destruction and recycling**

- Since Hyper Chrom Heparin HP Agarose chromatography media is difficult to degrade in nature, incineration of discarded chromatography media is recommended in order to protect the environment.
- For chromatography media exposed to bioactive samples such as viruses and blood, please follow local biosafety requirements before destroying or disposing of them.

Notes

1. It is recommended that the buffer and protein solution used for purification be filtered through a 0.22 μm or 0.45 μm membrane and then used on the column.
2. The chromatography medium was kept in 20% ethanol, 0.05 M sodium acetate solution with a glue suspension ratio of approximately 75%.
3. This product is for scientific purposes only.

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