HyperTrap DEAE FF Column

Product description

HyperChrom DEAE FF Agarose is a weak anion exchange chromatography medium that separates molecules using differences in charge properties and how much they are under specific conditions. It was found that when sodium hydroxide solution was continuously added dropwise to the chromatography medium, measuring the pH of the supernatant found that it could maintain a fairly wide range of pH stability. It is precisely because of this property that HyperChrom DEAE FF Agarose chromatography media exhibits an unexpected separation and purification effect. HyperChrom DEAE FF Agarose has excellent scale-up performance: 1) Highly crosslinked agarose base frame with excellent rigidity, so it can achieve high process flow rates and improve process efficiency at low back pressure. 2) Through chemical modification, it has excellent chemical compatibility and is resistant to CIP cleaning such as sodium hydroxide.

HyperTrap DEAE FF Column is based on HyperChrom DEAE FF Agarose as the chromatography medium for the isolation and purification of a variety of biomolecules, such as: recombinant proteins, antibodies, nucleic acids, viruses and virus-like particles, polysaccharides, etc.

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 DEAE FF 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℃ 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 DEAE FF Column chromatography media parameters

Name	Description
Chromatography media type	Weak anion exchange
Ligation	-O-CH_2CH_2–N^+ $(C_2H_5)_3H$
Scaffolding	Highly cross-linked agarose
Average particle size	90 μm
Ion load	0.11-0.16 mmolCl^-/mL chromatography medium
Dynamic load	> 90 mg OVALBUMIN/mL chromatography medium*
Flow rates are recommended	300-600 cm/h
Maximum flow rate	700 cm/h
Withstand pressure	0.3 MPa
Use temperature	4-30℃
pH stability**	2-14
Solvent resistant	Common aqueous solution, 30% isopropanol***, 75% ethanol***, 1 M NaOH, 1 M acetic acid, 6 M guanidine hydrochloride, 8 M urea
Intolerant to solvents	Oxidant, anionic detergent

* Dynamic load measurement conditions: column loading height: 10cm, test flow rate 300 cm/h; Test buffer: 0.05 M Tris-HCl solution, pH 8.0; test sample: 6 mg/ml OVALBUMIN sample, OVALBUMIN loading volume per unit volume (mL) (mg) when the penetration of OVALBUMIN reaches 10%.

**After 7 days in the environment of chromatography at 40℃ and pH 2-14, the physical and chemical properties and functions of the chromatography medium did not change significantly.

***30% and 75% are v/v, volume ratio.

Experimental manipulation

1. Preparation of buffers

Buffer selection: Buffer salts that do not interact with the chromatography medium should be selected.

- If the binding elution mode is used: low salt (less than 5 mS/cm) and high pH (usually 1 pH unit higher than the isoelectric point of the target molecule) buffer should be used for equilibration buffers to facilitate the binding of target molecules, and the stability of the sample in the buffer should be considered; Elution buffers are usually buffers in which a high concentration of salt (e.g., 1 M NaCl) is added to the equilibration buffer.
- If the flow through mode is used: the equilibration buffer should be treated under conditions

conducive to the binding of impurities, and rinsed directly with a high concentration of salt after the target molecule has completely flowed through.

2. **Sample preparation**

Prepare samples for purification.

3. **Chromatographic conditions**

- Flow rate selection: Linear flow rate of 300-600 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: Adequately equilibrate the column to pH and conductivity stable using equilibration buffer and is essentially the same as the equilibration buffer, which typically requires 3-5 times the column volume.

(2) Sample loading*: Determine the sample loading volume and amount on the HyperTrap DEAE FF Column based on the binding load measured in the pilot experiment.

(3) Washing*: Rinse the column with equilibration buffer or other suitable buffer 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. Fractional collection is performed on the eluted sample. pH gradient elution or mixed elution is also available.

(5) Regeneration: Rinse the column with buffer containing high salt (e.g., 2 M NaCl).

(6) Re-equilibration: Re-equilibrate the chromatography column with equilibration buffer.

*Note: * If the flow-through mode is used, the "sample loading" step should be set up for collection; The "washing" step ensures that after all the target molecules have flowed through, the collection can be stopped; In the "elute" step, the impurities are directly eluted with a high-salt 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 5x column volume of 2 M NaCl solution, or use a high salt buffer at pH 2 or higher, such as 1 M NaAc solution.
- Removal of strong hydrophobic proteins and precipitated proteins: first wash with 1 M NaOH solution in 5 column volumes, and then clean the lye with 5-10 column volumes of purified water.
- Removal of lipoproteins and lipids: Wash first with 5x column volume of 70% ethanol or 30% isopropanol, then rinse with 5-10x column volume of purified water.

  Note: 70% ethanol or 30% isopropanol should be degassed before use; The flow rate during the cleaning process can be selected from 30-60 cm/h; When the blockage is severe, 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℃.

10. **Destruction and recycling**

- Since HyperTrap DEAE FF Column chromatography media is difficult to degrade in nature, incineration of discarded chromatography media is recommended 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. All chromatography media are kept in 20% ethanol solution with a glue suspension ratio of approximately 75%.

3. This product is for scientific purposes only.

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