HyperChrom DEAE HP Agarose

Product description

HyperChrom DEAE HP 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 HP Agarose chromatography media exhibits unexpected separation and purification effects. The chromatography medium can be well used for the isolation and purification of a variety of biomolecules, such as: recombinant proteins, antibodies, nucleic acids, viruses and virus-like particles, polysaccharides, etc.

HyperChrom DEAE HP 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. (3) Fine particle size design to improve resolution.

Components and storage conditions

Components	PC2013-25 mL	PC2013-100 mL
HyperChrom DEAE HP Agarose	25 mL	100 mL
Store the components at 4℃ for 5 years.		

Product parameters

HyperChrom DEAE HP Agarose chromatography media parameters

Name	Description
Chromatography media type	Strong anion exchange
Ligation	-O-CH_2CH_2–N^+ $(C_2H_5)_3H$
Scaffolding	Highly cross-linked agarose
Average particle size	34 μm
Ion load	0.08-0.13 mmol Cl^-/mL chromatography medium
Dynamic load	>90 mg ovalbumin/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 **	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 150 cm/h; Test buffer: 0.05 M Tris-HCl solution, pH 8.0; test sample: 6 mg/ml BSA sample, BSA loading volume per volume of media (mL) (mg) when BSA penetration reaches 10%.

**After 7 days in the environment of chromatography at 40 °C 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 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. Loading columns

The following column loading methods are suitable for filling laboratory-scale chromatography columns:

4.1 Supplies required for column mounting

(1) Chromatography medium: HyperChrom DEAE HP Agarose

(2) Chromatographic empty column: laboratory-scale chromatography empty column and column loader

(3) Solution required:

a) Column loading solution: purified water.

b) Exhaust solution: purified water.

(4) Column loading tools: sand core funnel, stirring rod, measuring cylinder, etc.

4.2 Preparation before column loading

(1) Calculate the volume of chromatography medium Vm required for column loading (volume of the chromatography medium part after sufficient sedimentation), and calculate the formula:

Vm = cross-sectional area of the chromatography column X height of the column bed where the column is planned to be loaded x compression ratio of the chromatography medium.

(Note: HyperChrom DEAE HP Agarose has a compression ratio of 1.12).

(2) The chromatography medium was transferred to the sand core funnel, and the column loading solution was cleaned and filtered with about 3 times the volume of the chromatography medium, and the column chromatography medium to be loaded was replaced with the column loading solution.

(3) To prepare the glue suspension of the column chromatography medium to be loaded, the suitable column loading glue suspension ratio of HyperChrom DEAE HP Agarose chromatography medium is 50%-60%. In order to obtain an accurate chromatography medium volume, the chromatography medium can be placed in a graduated cylinder and settled overnight or centrifuged at low speed (3000 rpm, 5 min) to simulate the natural sedimentation effect of the chromatography medium, and then measured.

(4) Check the empty column to be used to ensure it is clean and leak-free.

4.3 Column mounting

(1) Exhaust the column bottom membrane (screen) with purified water.

(2) After sufficient exhaustion, screw the plug or close the column bottom valve at the bottom of the column interface, and continue to inject a small amount of purified water until the bottom of the

column is covered.

(3) Adjust the chromatography column to vertical.

(4) The column head is connected to the chromatography system, which provides a low flow rate of 5 m L/min through the chromatography system, and exhausts the column head membrane (screen) with purified water.

(5) Thoroughly stir the prepared chromatography medium suspension with a stir bar, and then slowly pour into the prepared chromatography empty column at one time.

Note: If the volume of the glue suspension exceeds the empty column volume, it should be extended by using a column loader or connecting another empty column tube with a connector.

(6) Place the gaseated stigma into the chromatography column, fully fit the glue suspension level, and remove all air bubbles. Then tighten the column head seal.

(7) Start the system pump, control the pressure at 0.3 MPa, and use the liquid flow to compress the column bed. During this period, the pressure should not exceed 0.3 MPa. If the pressure is over, the flow rate needs to be reduced.

CID / V/FR / LFR	10 mm	16 mm	26 mm	50 mm
60 cm/h	0.8 mL/min	2.0 mL/min	5.3 mL/ min	19.6 mL/min
100 cm/h	1.3 mL/min	3.3 mL/min	8.8 mL/min	32.7 mL/min
1 50 cm/h	2.0 mL/min	5.0 mL/min	13.3 mL/ min	49.1 mL/min
200 cm/h	2.6 mL/min	6.7 mL/min	17.7 mL/ min	65.4 mL/min
300 cm/h	3.9 mL/min	10.0 mL/min	| 26.5 mL/min	98.1 mL/min
600 cm/h	7.9 mL/min	20.1 mL/ min	53.1 mL/min	196.3 mL/ min

Note:
CID: Chromatographic inner diameter
V/FR: Volumetric flow rate
LFR: Linear flow rate

Table 1 Flow rate conversion table of different specifications of chromatography columns

(8) After the column bed is stabilized (the glue surface no longer falls), mark the position of the glue surface at this time. Stop the pump and press the column head down to 2-3 mm below the marked position.

(9) Re-apply the flow rate of 600 cm/h, if the glue surface no longer drops, that is, the column loading is completed. If the glue surface falls, repeat steps 8-9.

Note: The recommended workflow speed does not exceed 75% of the flow rate of the loading column.

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 HyperChrom DEAE HP Agarose 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 2 M NaCl solution in 5x column volumes, 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 °C.

10. Destruction and recycling

- Since HyperChrom DEAE HP Agarose 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.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com