HyperTrap Butyl HP Column

Product description

HyperChrom Butyl HP Agarose is a hydrophobic interaction chromatography medium that takes advantage of differences in the hydrophobic properties and the amount of different molecules under specific conditions. HyperChrom Butyl HP Agarose has excellent scale-up performance: 1) Highly crosslinked agarose base with excellent rigidity, so it can achieve high process flow rates and improve process efficiency at low back pressure. 2) Hydrophilic base frame to minimize the influence on the hydrophobic effect of the ligand. 3) Through chemical modification, it has excellent chemical compatibility and is resistant to CIP cleaning such as sodium hydroxide. 4) Fine particle size design to improve resolution.

HyperTrap Butyl HP Column is a preloaded column with HyperChrom Butyl HP Agarose as the chromatography medium, which is mainly used for the isolation and purification of a variety of biomolecules, such as recombinant proteins, antibodies, viral vaccines, 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 Butyl 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 Butyl HP Column chromatography media parameters

Name	Description
Chromatography media type	Hydrophobic interactions
Ligation	R-O-CH_2-CH(OH)-CH_2-O-$(CH_2)_3$-CH_3
Scaffolding	Highly cross-linked agarose
Average particle size	34 μm
Ligand density	~50 μmol Butyl group/mL chromatography medium
Dynamic load	~38 mg β-lactoglobulin/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*	3-13
Solvent resistant	Common aqueous solution, 3 M ammonium sulfate, 30% isopropanol**, 75% ethanol**, 1 M NaOH, 1 M acetic acid, 6 M guanidine hydrochloride, 8 M urea
Intolerant to solvents	Oxidant
* After the chromatography medium was placed at 40 °C and pH 3-13 for 7 days, its physicochemical properties and functions 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 binding elution mode is used: a high-salt buffer (e.g., a buffer containing 1.5 M ammonium sulfate) should be used for the balance buffer to facilitate the binding of the molecule of interest, while considering the stability of the sample in the buffer; the elution buffer is usually a buffer with high salt removed from the equilibration buffer.
- If the flow mode is used: the equilibration buffer should be under conditions conducive to the binding of impurities, and rinse directly with a low concentration salt after the target molecule is completely flowed.

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: Adequately equilibrate the column to pH and conductivity stable using equilibration buffer and is substantially consistent with equilibration buffer, a step that typically requires 3-5x column volume.

(2) Sample loading*: Determine the sample loading volume and amount on the HyperTrap Butyl HP 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 reducing the salt ion concentration, which can gradually reduce the salt ion concentration in the eluate through a linear gradient or step gradient, and 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 chromatography column with buffer containing low salt.

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

*Note: * If the flow-through mode is used, the "loading" step should set up collection, the "washing" step should ensure that the target molecules have flowed through, and the "elution" step can be used to directly elute the impurities with 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 purified water.
- 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 can be selected from 30-60 cm/h during the cleaning process; reverse cleaning can be used when the blockage is serious.

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**

   For unopened chromatography media, please store in the original container; the completed chromatography column should be soaked with 20% ethanol solution before closing the upper and lower column heads. The storage environment is 4~30 °C.

10. **Destruction and recycling**

    - Since HyperTrap Butyl HP 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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