HyperTrap Protein G 4FF Column

Product description

HyperChrom Protein G 4FF Agarose is an affinity chromatography medium made by coupling Protein G to a substrate by CNBr activation. Protein G can bind to the Fc region of IgG of a variety of mammals and also has a weak affinity for its Fab region. Compared to Protein A, Protein G has a broader binding spectrum. Therefore, HyperChrom Protein G 4FF Agarose can be used to isolate and purify immunoglobulins and their fragments from different liquid samples such as cell culture media or serum. HyperChrom Protein G 4FF Agarose can also be used to isolate immune complexes.

HyperTrap Protein G 4FF Column is a ready-to-use chromatography column with HyperChrom Protein G 4FF Agarose as the packing material, which has the advantages of high flow rate, high load capacity, easy regeneration, low cost and easy amplification.

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 Protein G 4FF 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 Protein G 4FF Column chromatography media parameters

Name	Description
Chromatography media type	Affinity chromatography media
Ligation	Protein G (E. coli recombinant expression).
Scaffolding	Highly cross-linked agarose
Average particle size	90 μm
Ligand density	~2 mg Protein G/mL chromatography medium
Dynamic load	≥ 20 mg human IgG/mL chromatography medium*
Flow rates are recommended	90-300 cm/h
Maximum flow rate	400 cm/h
Withstand pressure	0.3 MPa
Use temperature	4-30℃
pH stability **	3-10
Chemical resistance	Common aqueous solution, 30% isopropanol, 75% ethanol, 6 M guanidine hydrochloride, 8 M urea

*Dynamic load measurement conditions: column loading height: 10 cm, test flow rate 200 cm/h, test buffer: 0.02 M NaH_2PO_4 solution, pH 7.0, test sample: 2 mg/m IgG sample, when the penetration of IgG reaches 10%, unit medium volume (mL) IgG loading volume (mg).

**After 7 days of storage in the environment of 40 °C and pH 3-9, the physicochemical properties and functions of the chromatography medium did not change significantly.

*** 30% and 75% are the volume ratio (v/v).

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	0.1 M glycine, pH 2.5-3.0

2. Sample preparation

Prepare samples for purification.

3. Chromatographic conditions

- Flow rate selection: Linear flow rate of 90-300 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 microporous membrane (after inclusion body disruption) before loading, and it is recommended that the pH and conductivity of the sample be adjusted to be consistent with the equilibration 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, Wh = 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 loading volume on the HyperTrap Protein G 4FF Column 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 Buffer is lowered for washing. Immediately after elution, the collection components should be neutralized with 1 M Tris-HCl, pH 9 buffer, which can be added at a ratio of 60-200 µl to 1 ml of the elution collection.

(5) Re-equilibration: Re-equilibrate the chromatography column with a 10x volume 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:

- Method 1: To remove strong hydrophobic proteins, lipoproteins and lipids, etc., it can be treated with 0.1% nonionic detergent at 37 °C with a contact time of 1 min, and then rinsed with at least 5 column volumes of conjugate solution.
- Method 2: Soak in 70% ethanol for 12 h to remove lipids, then rinse with at least 5 column volumes of binding solution.

8. sterilization

To reduce the microbial load, 20% ethanol was used for more than 6 h to achieve sterilization.

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 2~8 °C.

10. Destruction and recycling

- Since HyperTrap Protein G 4FF 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. This product is for scientific purposes only.

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