HyperTrap AR rProtein A/G 4FF Column

Product description

HyperTrap AR rProtein A/G 4FF Column is an affinity chromatography z-packed column for antibody purification that is separated by a specific interaction between antigen and antibody, mainly for the purification of IgG in culture fluid, serum, ascites, or hybridoma cell culture supernatant, or the purification of recombinant proteins containing Fc fragments.

HyperChrom AR rProtein A FF Agarose and HyperChrom Protein G 4FF Agarose in this product are covalently connected to 4% high crosslinked, high flow rate agarose and mixed in a 1:1 ratio. Each milliliter of HyperChrom AR rProtein A/G Agarose 4FF Agoros can bind approximately 20 mg of human IgG.

Product parameters

1. HyperTrap AR rProtein A/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℃ 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 AR rProtein A/G 4FF Column chromatography media parameters

Name	Description
Chromatography media type	Affinity chromatography media
Ligation	rProtein A/G (E. coli recombinant expression, 1:1).
Scaffolding	Highly cross-linked agarose
Average particle size	90 μm
Dynamic load	~20 mg human IgG/mL chromatography medium
Flow rates are recommended	50-400 cm/h
Withstand pressure	0.3 MPa
Use temperature	4-30℃
pH stability	3-9

Experimental manipulation

1. **Preparation of buffers**

Buffer Type	Buffer Components
Balance/Bind/Wash Buffer	PBS pH 7.4
Elution Buffer	100mM glycine, pH 2-3
Neutralization buffer	1M Tris-HCl, pH 8.8

2. **Sample preparation**

Prepare samples for purification.

3. **Chromatographic conditions**

- Flow rate selection: a linear flow rate of 50-400 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) Dilution: To ensure that the sample solution has the appropriate ionic strength and pH value, dilute the serum, ascites or cell culture medium samples with Balance/Bind/Wash Buffer at least 1:1 ratio before going to the column; Samples can also be placed in dialysis bags with a molecular weight of 3.5 kDa and dialyzed overnight with Balance/Bind/Wash Buffer in a 4°C freezer. *[Note]: Plasma samples may be turbid due to lipoprotein precipitation in plasma during the dilution process, only 10,000 g centrifugation for 20 minutes to take the supernatant.*

(2) 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.

(3) Sample loading: Determine the sample loading volume and amount on the HyperTrap AR rProtein A/G 4FF Column based on the binding load measured in the pilot experiment.

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

(5) Elution: Elution is performed by lowering the pH of Elution Buffer, and the eluate is balanced by adding an appropriate amount of Neutralization buffer. *Note: Samples can then be preserved according to the purpose of use or the collected protein is dialyzed into its purpose of storage.*

(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:

- 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**

In order 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 AR rProtein A/G 4FF Column chromatography media is difficult to degrade in nature, it is recommended to incinerate the waste chromatography media 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. This product is for scientific purposes only.

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