HyperChrom AR rProtein A/G Agarose 4FF Agarose

Product description

HyperChrom AR rProtein A/G Agarose 4FF Agarose is an affinity chromatography medium for antibody purification, separated by specific interaction between antigens and antibodies, mainly used for the purification of IgG in culture media, serum, ascites or hybridoma cell culture supernatant, or the purification of recombinant proteins containing Fc fragments.

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

Components and storage conditions

Components	PC2007-25 mL	PC2007-**100 mL**
HyperChrom AR rProtein A/G Agarose 4FF Agarose	25 mL	100 mL
Store the components at 4℃ for 5 years.		

Product parameters

HyperChrom AR rProtein A/G Agarose 4FF Agarose 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. 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 AR rProtein A/G Agarose 4FF Agarose

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

(3) Solution required:

a) Column loading solution: 20% ethanol.

b) Exhaust solution: 20% ethanol.

(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: The compression ratio of HyperChrom AR rProtein A/G Agarose 4FF Agarose is 1.15).

(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) For the preparation of the gel suspension of the column chromatography medium to be loaded, the suitable column loading gel suspension ratio of HyperChrom AR rProtein A/G Agarose 4FF Agarose chromatography medium is 45%-55%. 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) Vent the column bottom membrane (screen) with 20% ethanol.

(2) After sufficient exhaust, screw the plug or close the column bottom valve at the bottom interface of the column, and continue to inject a small amount of 20% ethanol 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, through which a low flow rate of 5 m L/min is provided, and the column head filter membrane (screen) is vented with 20% ethanol.

(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, adjust the flow rate to 600 cm/h, and use the liquid flow to press 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 (see the table below for flow rate conversion).

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 currently. Stop the pump and press the column head down to 3-5 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) 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 HyperChrom AR rProtein A/G Agarose 4FF Agarose 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 37ºC 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 2~8ºC.

10. **Destruction and recycling**

- Since HyperChrom AR rProtein A/G Agarose 4FF Agarose 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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