HyperChrom AR rProtein A FF Agarose

Product description

HyperChrom AR rProtein A FF Agarose is an affinity chromatography medium for antibody purification, separated by specific interaction between antigen and antibody, mainly used to purify monoclonal and polyclonal antibodies.

HyperChrom AR rProtein A FF Agarose has high selectivity, high yield, and easy to scale up properties: (1) recombinant protein A is produced in E. coli and engineered for directional conjugation to enhance binding load; Epoxy-based conjugation ensures low ligand shedding; recombinant protein A has a specificity similar to native protein A to the LgG Fc region, which improves selectivity and yield. (2) Process amplification is relatively simple.

Components and storage conditions

Components	PC2005-25 mL	PC2005-100 mL
HyperChrom AR rProtein A FF Agarose	25 mL	100 mL
Store the components at 4℃ for 5 years.		

Product parameters

HyperChrom AR rProtein A FF Agarose chromatography media parameters

Name	Description
Chromatography media type	Affinity chromatography media
Ligation	rProtein A (E. coli recombinant expression)
Scaffolding	Highly cross-linked agarose
Average particle size	90 μm
Ligand density	6 mg rProtein A/ml chromatography medium
Dynamic load	~35 mg human IgG/mL chromatography medium*
Flow rates are recommended	150-500 cm/h
Maximum flow rate	700 cm/h
Withstand pressure	0.3 MPa
Use temperature	4-30℃
pH stability **	3-10
Chemical resistance	6 M guanidine hydrochloride, 8 M urea, 2% benzyl alcohol, 75% ethanol, 1 mM NaOH (pH11), 0.1 M sodium citrate/HCl (pH3).
** Dynamic load measurement conditions: column loading height: 5 cm, retention time 3 min, test buffer:		

0.02 M NaH_2PO_4 solution, pH 7.0, test sample: human lgG sample, lgG sample per unit media volume (ml) when the penetration of lgG reaches 10%.

**After 7 days of storage of chromatography medium at 40°C and pH 3-10, its physicochemical properties and functions did not change significantly.

***30% and 75% are v/v, volume ratio.

Experimental manipulation

1. **Preparation of buffers**

Buffer type	Buffer components
Balance/Bind/Wash Buffer	0.15 M NaCl，20 mM Na2HPO4，pH7.0
Elution Buffer	50 mM Na2HPO4, pH 3.0 or 50 mM sodium citrate, pH 3.0

2. **Sample preparation**

Prepare samples for purification.

3. **Chromatographic conditions**

- Flow rate selection: Linear flow rate of 150-500 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 FF 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) To calculate the volume of chromatography medium required for column loading Vm (volume of the chromatography medium part after sufficient sedimentation), the calculation 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 FF Agarose is 1.15).

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

(3) For the preparation of the gel suspension of the column chromatography medium to be loaded, the suitable proportion of column loading glue suspension for HyperChrom AR rProtein A FF Agarose chromatography medium is 45%-55%. 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) 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, which provides a low flow rate of 5 m L/min through the chromatography system, and exhausts the column head filter membrane (screen) 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 column head 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 at this time. 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 does not drop again, 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, 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 amount on the HyperChrom AR rProtein A FF Agarose 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.

(5) 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., 0.1% nonionic detergent can be treated 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**

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 2~8ºC.

10. **Destruction and recycling**

- Since HyperChrom AR rProtein A FF Agarose chromatography media is difficult to degrade in nature, incineration of discarded chromatography media is recommended 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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