HyperChrom Glutathione 4FF Agarose

Product description

HyperChrom Glutathione 4FF Agarose is an affinity chromatography medium primarily used to purify glutathione S-transferase (GST)-tagged proteins, but also for the purification of other S-transferases or glutathione-dependent proteins. HyperChrom Glutathione 4FF Agarose is the first choice for purifying GST-tagged proteins, with the advantages of high loading, easy regeneration, and easy amplification.

Components and storage conditions

Components	PC2003-25 mL	PC2003-100 mL
HyperChrom Glutathione 4FF Agarose	25 mL	100 mL
Store the components at 4℃ for 5 years.		

Product parameters

HyperChrom Glutathione 4FF Agarose chromatography media parameters

Name	Description
Chromatography media type	Affinity chromatography media
Ligation	Glutathione and 10 carbon atoms connect arms
Scaffolding	4% highly cross-linked agarose
Average particle size	90 μm
Ligand density	120~320 μ mol glutathione/mL chromatography medium
Dynamic load	≥ 10 mg GST-tagged protein/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 - 12
Chemical resistance	Common aqueous solution, 0.01 M HCl, 0.1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol **

* After the chromatography medium was placed at 40 °C and pH 3-12 for 7 days, its physicochemical properties and functions did not change significantly.

** 30% is v/v, volume ratio.

Experimental manipulation

1. Preparation of buffers

Buffer Type	Buffer Components
Balance/Bind/Wash Buffer	PBS, pH 7.3 (140 mM NaCl, 2.7 mM KCl, 10 mM Na 2 HPO 4 , 1.8 mM KH 2 PO 4 , pH 7.3)
Elution Buffer	50 mM Tris-HCl, 10 mM Reduced Glutathione, pH 8.0

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 (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: Hpyer Chrom Glutathione 4FF Agarose.

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

(3) Solution required:

a) Column loading solution: PBS, pH 7.3.

b) Exhaust solution: PBS, pH 7.3.

(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 Hpyer Chrom Glutathione 4FF Agarose is 1.12).

(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 proportion of column loading glue suspension for HyperChrom Glutathione 4FF Agarose chromatography medium is 50% - 70%. 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) Exhaust with PBS, pH 7.3 for the column bottom filter membrane (screen).

(2) After sufficient exhaust, screw the plug or close the column bottom valve at the bottom of the column interface, and continue to inject a small amount of PBS, pH 7.3, 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 and exhausts the column head membrane (screen) with PBS, pH 7.3.

(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 300 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 2-3 mm below the marked position.

(9) Re-apply the flow rate of 300 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 > 5000, 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: 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 sample loading amount on the HyperChrom Glutathione 4FF 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: Elute the binding protein with 5-10 column volumes of elution buffer.

(5) Rebalancing: Re-equilibrate the chromatography column with Balance/Bind/Wash Buffer.

7. Removal of GST tags

(1) Excision of GST tags with PreScission enzyme

- PreScission enzyme
- PreScission buffer：50 mM Tris-HCl，50 mM NaCl，1 mM EDTA，1 mM DTT，pH 7.5

Note: Digestion is performed prior to the chromatography step (4-elution).

The specific process is:

A. Rinse the chromatography column with 10x column volume of PreScission digestion buffer.

B. PreScission enzyme mix preparation: Prepare 80 μL of PreScission enzyme and 920 μL of PreScission digestion buffer per ml of chromatography medium (8 mg target protein/mL chromatography medium), 4°C.

C. Load the PreScission enzyme mixture into a chromatography column, block the chromatography column, and incubate at 4°C for 4 h.

D. Rinse the chromatography column with 3x column volume of PreScission digestion buffer and collect the eluate with different tubes containing the protein of interest and the PreScission

enzyme.

(2) Excision of the GST tag with Thrombin enzyme

- Thrombin enzyme
- Thrombin digestion buffer: PBS, pH 7.3

Dissolve 500 U of Thrombinase in 500 μL PBS, pH 7.3 (1 U/μL), aliquot into 80 μL per tube, and freeze at -80°C.

Note: Digestion is performed prior to the chromatography step (4-elution).

The specific process is:

A. Thrombinase Mixture Preparation: Prepare 80 μL of Thrombinase and 920 μL of Thrombin digestion buffer per ml of chromatography medium (8 mg protein of interest/ml chromatographic medium).

B. The thrombin enzyme mixture was loaded into the chromatography column, the chromatography column was blocked, and the room temperature (22~25°C) was incubated for 2~16 h.

C. Rinse the chromatography column with 3x column volume of Thrombin digestion buffer and collect the elution with different tubes containing the protein of interest and Thrombinase.

(3) Remove the GST tag with Factor Xa

- Factor Xa *[Note] Factor Xa consists of two subunits linked by disulfide bonds, and since glutathione can break disulfide bonds, glutathione needs to be removed from the sample prior to the excision reaction.*
- Factor Xa buffer：50 mM Tris-HCl, 150 mM NaCl, 1mM $CaCl_2$, pH7.5。

Dissolve 400 U Factor Xa in 400 μL of cold water (1 U/μL), aliquot into 80 μL per tube, and freeze at -80°C.

Note: Tag excision is performed prior to the chromatography step (4-elution).

The specific process is:

A. Rinse the column with 10x column volume of Factor Xa excision buffer.

B. Factor Xa mixture preparation: Prepare 80 μL of Factor Xa and 920 μL of Factor Xa excision buffer per mL of chromatography medium (8 mg target protein/mL chromatography medium).

C. Load the Factor Xa mixture into the chromatography column, block the chromatography column, and incubate for 2~16 h at room temperature (22~25°C).

D. Rinse the chromatography column with 3x the column volume of Factor Xa excision buffer

and collect the eluate with a different tube containing the protein of interest and Factor Xa.

8. 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).

- Removal of denaturing and precipitated proteins: first wash with 2x column volume of 6 M guanidine hydrochloride, then rinse with 5x column volume PBS, pH 7.3.
- Removal of hydrophobic and lipids: Wash the chromatography column first with 3-4 column volumes of 70% ethanol, then with 5 column volumes of PBS, pH 7.3.

9. Destruction and recycling

- Since hpyer Chrom Glutathione 4FF 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.

10. stockpile

The preloaded column is stored at the factory with 20% ethanol and closed with upper and lower plugs to keep the chromatographic packing wetted. After opening and using the preloaded column, the user can first rinse the preloaded column with pure water, then rinse 2-3 times the column volume with 20% ethanol, and then seal it with a plug (solution volatilization will cause the chromatographic packing to dry out and shrink, affecting its separation performance). For unopened chromatography media, keep in the original container.

11. frequently asked questions

issue	Possible causes	Recommend a solution
The column backpressure is too high	The packing is blocked	The lysate may contain tiny solid particles, and it is recommended to filter with a membrane (0.22 μm or 0.45 μm) before loading the column, or centrifuge for removal
		Samples contain high concentrations of nucleic acids, extend the disruption time until the viscosity decreases, or add DNase I (final concentration 5

		μg/mL), Mg2+ (final concentration 1 mM), and incubate on ice for 10-15 min
	The sample is too viscous	Organic reagents or protein-stabilizing agents (e.g., glycerol, etc.) may cause increased back pressure and decrease the operating flow rate.
There is no protein of interest in the eluting component	Proteins may be inclusion bodies	The lysate can be detected by electrophoresis to analyze whether there is a protein of interest in the supernatant, and the inclusion body protein needs to be purified according to the inclusion body protein
	The expression is too low	Optimize the expression conditions and use the inclusion body purification buffer system
	The target protein binding is relatively weak and has been washed down during the washing step	Increase the pH of the Wash Buffer, or decrease the concentration of imidazole
	The protein of interest binds too strongly and is not easy to elute off	Lower the pH of Elution Buffer, or increase the concentration of imidazole in Elution Buffer
		Peel off nickel ions using 10-100 mM EDTA solution while obtaining proteins
	Protein degradation	When the bacteria are broken, some protease inhibitors need to be added
		Perform the purification operation at 4°C
Impure elution components (containing multiple proteins)	Incomplete washing	Increase the Wash Buffer volume
	The sample contains other His-tagged proteins	Optimize washing conditions by adjusting pH or imidazole concentration. The eluting components were further purified using other purification methods (e.g., deionization exchange, hydrophobicity, etc.).
The filler color becomes lighter or turns white	Nickel ions shed or peeled off	Re-hang nickel ions according to the operation of filler regeneration
The filler is brown	The buffer contains reducing agents such as	Appropriately reduce the concentration of reducing agent DTT, or switch to thioethanol

	DTT	
Protein precipitation occurs during sample loading	The operating temperature is too low	Load samples at room temperature
	Proteins aggregate	Add stabilizer to the sample and all buffers, such as 0.1% Triton X-100 or Tween-20

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. Sterilization: Since the 20% ethanol preservation solution does not have the effect of sterilization and pyrogen removal, it is recommended that HyperChrom Glutathione 4FF Agarose media can be treated with 70% ethanol for more than 12 h before and during use, or the denickelized medium can be treated with 1 M NaOH for 0.5~1 h to reduce the risk of microbial contamination.
3. This product is for scientific purposes only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com