HyperTrap Glutathione 4FF Column

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. HyperTrap Glutathione 4FF Column is a ready-to-use chromatography column with HyperChrom Glutathione 4FF Agarose as the filler, which can be used to purify histidine-tagged proteins, with high flow rate, high load, easy regeneration, low cost, easy amplification and so on.

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 Glutathione 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 Glutathione 4FF Column 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. **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 > 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 HyperTrap Glutathione 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: 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 DTT	Appropriately reduce the concentration of reducing agent DTT, or switch to thioethanol
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 HyperTrap Glutathione 4FF Column 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.

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