HyperTrap Ni-NTA Excel Column

Product Description:

HyperChrom Ni-NTA Excel Agarose is an affinity chromatography medium that chemically couples a tetracoordinated transition metal ion Ni^{2+} to a ligand of Nitrilotriacetic acid (NTA) using a highly cross-linked agarose gel as a matrix. It has a very stable octahedral structure, with nickel ions in the center of the octahedron to protect nickel ions from small molecules, is more stable, and can withstand harsh conditions such as reducing agents, denaturants or couplants at certain concentrations. In addition, due to the pressure resistance of the matrix, which can withstand pressures up to 0.3 MPa, the product can be used for industrial-scale protein purification, enabling purification of the protein of interest at relatively high flow rates. This chromatography medium uses the interaction between Ni^{2+} and the side chains of certain amino acids (mainly histidine) on the protein to separate and purify proteins with and without these amino acids, and with or without these amino acids.

HyperTrap Ni-NTA Excel ColumnReady-to-use columns with HyperChrom Ni-NTA Excel Agarose can be used to purify histidine-tagged proteins with tolerances such as 100 mM EDTA and 0.5 M NaOH immersion for 48 h.

Product parameters

1. HyperTrap Ni-NTA Excel Column preloaded column parameters

Prepacked column specifications/names	1 mL	5 mL
Dimensions (IDxH)	7.7 x 25 mm	16 x 25 mm
Pressure-resistant	0.3 MPa	0.3 MPa
Workflow speed*	1 mL/min	1-3 mL/min
Operating temperature	4 - 30℃	4 - 30℃
Storage conditions	Store the components at 4℃ for 5 years.	
*This flow rate is the recommended common flow rate, the pressure resistance, and the flow rate of different types of chromatography resins in the prepacked column will be different, please refer to the product manual of the corresponding chromatography resin.		

2. HyperTrap Ni-NTA Excel Column chromatography media parameters

Name	Description
Chromatography media type	Affinity chromatography media
Ligands	NTA-Ni^{2+}

Scaffolding	Highly cross-linked agarose
Average particle size	90 μm
Ligand density	54~70 μmol Ni^{2+}/mL chromatography medium
Dynamic load	≥ 10 mg histidine-tagged protein/mL chromatography medium
Flow rate is recommended	150-600 cm/h
Maximum flow rate	600 cm/h
Pressure-resistant	0.3 MPa
Operating temperature	4 - 30℃
pH stability*	2-14
Chemical resistance	■ 0.1 M~0.5 M NaOH for 48 h ■ 10 mM β-mercaptoethanol, 5 mM TCEP for 24 h ■ 500 mM imidazole, 100 mM EDTA, 2 h
*After 7 days of chromatography media at 40°C, pH 2-14, there was no significant change in its physicochemical properties and functions.	

Experimental manipulation

1. Preparation of buffers

Buffer type	Buffer components
Balance/Bind Buffer	0.02 M PB, 0.5 M NaCl, pH 7.4
Wash Buffer	0.02 M PB, 0.5 M NaCl, 0-30 mM imidazole, pH 7.4
Elution Buffer	0.02 M PB, 0.5 M NaCl, 500 mM imidazole, pH 7.4
Store Buffer	20% ethanol, store at 4-30 °C

2. Chromatography conditions

- Flow rate selection: According to the height of the column bed, a linear flow rate of 150-600 cm/h is generally selected.
- Sample preparation: To prevent the sample from clogging the column, the sample is filtered with a 0.2/0.45 μm (after inclusion body breakage) microporous membrane prior to loading, and it is recommended that the pH and conductivity of the sample be adjusted to be consistent with the equilibrium buffer (dilution, ultrafiltration, and desalting can be used to adjust the pH and conductivity of the sample).

3. Pre-packed column settings

1) Open the package and remove the pre-packed column.

2) Connect the prepacked column: Unscrew the upper and lower plugs of the prepacked column and connect to the chromatography system (peristaltic pump or syringe, etc.), and rinse the prepacked column with pure water 2-3 times the column volume to drain the preservation solution (typically 20% ethanol). To prevent air bubbles from entering during the connection, the connector can be

connected by dripping pure water into the inlet end of the prepacked column and filling it before connecting the system connector.

3) Wash and sterilize: For the first use, it is recommended to wash and disinfect the column and rinse 2 times the column volume with pure water or buffer. Cleaning and disinfection buffers are recommended, please refer to the subsequent cleaning and regeneration steps.

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 prior to sample loading.

4. Column efficiency determination (optional).

Select one of the two test methods shown in the table below for column efficiency testing. The mobile phase equilibration column was used to stabilize the baseline, the sample was loaded into the column, the mobile phase was continued to be rinsed, and the chromatographic peaks were returned to the baseline after the operation was completed, and the chromatographic peaks were integrated to evaluate the loading effect.

Table2 Statistical table of the two column efficiency determination 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
Detectors	UV 280 nm	electrical conductivity

The main evaluation criteria for column loading effect 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 standard: N/m > 5000, 0.8 < As < 1.5

*Parameter Notes:

L= column height, V_R = reserved volume, W_h = peak width at half height, a = width at 10% peak height on the left half, b = width at right half height at 10% peak height.

5. Chromatography steps

1) Equilibration: Equilibrate the column well with Balance/Bind Buffer to pH and conductivity stability and essentially the same as equilibration buffer, a step that typically requires 3-5 column volumes.

2) Loading: Determine the sample loading volume and sample volume on the HyperTrap Ni-NTA Excel Column based on the binding capacity measured by the small-scale experiment.

3) Wash impurities: Wash Buffer or other appropriate buffers to flush the column until the UV is stable and returns to baseline.

4) Elution: Elution is achieved by increasing the concentration of imidazole, which can be achieved by gradually increasing the concentration of imidazole in the eluate through a linear gradient or a step-by-step gradient to elute molecules with different binding strengths.

5) Re-equilibration: Re-equilibrate the column with Balance/Bind Buffer.

6. Cleaning and regeneration

As the chromatography media is used, contaminants (e.g., lipids, endotoxins, proteins, etc.) accumulate on the column. Regular, cleaning-in-place is essential to keep the column in stable working order. The frequency of CIP is determined based on the degree of contamination of the chromatography media (if the contamination is severe, it is recommended that CIP should be performed after each use to ensure reproducibility of results and extend the working life of the chromatography media).

For different types of impurities and contaminants, cleaning can be carried out under the following conditions:

- Removal of bound proteins due to strong ion adsorption: Wash with 5 column volumes of 1.5 M NaCl solution.
- Removal of denatured, precipitated proteins, hydrophobic binding proteins, and lipids: Wash the column with 1 M NaOH for 1–2 h (12-24 h for endotoxin removal), then rinse the column with 10 column volumes of Balance/Bind Buffer.

Note: The flow rate can be selected from 30-60 cm/h during cleaning-in-place, and reverse cleaning can be used when the blockage is severe.

7. Destruction & Recycling

- Since Ni Chromstar FF chromatography media is difficult to degrade in nature, it is recommended to incinerate the waste chromatography media to protect the environment.
- For chromatography media that have meet biologically active samples such as viruses and blood, please follow local biosafety requirements before destroying or disposing of them.

8. Stockpile

The prepacked column is stored in 20% ethanol at the factory and closed with upper and lower plugs to keep the chromatography resin wet. After the user has opened and used the prepacked column, the prepacked column can be rinsed with pure water, then rinsed with 20% ethanol to rinse 2-3 times the

column volume, and then sealed with a plug (the volatilization of the solution will cause the chromatography resin to dry out and shrink, affecting its separation performance). Unopened chromatography media in the original container.

9. Frequently asked questions, FAQs.

Issues	Possible causes	Recommend solutions
The column backpressure is too high	The filler is clogged	The lysate may contain tiny solid particles, which are recommended to be filtered with a membrane (0.22 μm or 0.45 μm) or centrifuged prior to loading
		Samples containing high concentrations of nucleic acids were incubated for 10–15 minutes on ice with the addition of DNase I (final concentration of 5 μg/mL) and Mg^{2+} (final concentration of 1 mM).
	The sample is too viscous	Organic reagents or protein stabilization reagents (e.g., glycerol) may cause an increase in backpressure and reduce the flow rate of the operation.
There is no protein of interest in the elution 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 level is too low	Expression conditions were optimized, and inclusion bodies were used to purify the buffer system
	The binding of the protein of interest is relatively weak and has been washed off during the washing step	Increase the pH of Wash Buffer or decrease the concentration of imidazole
	The protein of interest binds too strongly and is not easy to elute	Reduce the pH of Elution Buffer, or increase the concentration of imidazole in Elution Buffer
		Use a 10-100 mM EDTA solution to strip the nickel ions and obtain the protein at the same time

	Protein degradation	Some protease inhibitors need to be added when the cell is broken
		Perform the purification operation at 4 °C
Impure elution components (containing a variety of proteins)	The washing is not thorough	Increase the volume of Wash Buffer
	Other His-tagged proteins were included in the sample	Wash conditions are optimized by adjusting the pH or imidazole concentration. The eluting components are then further purified using other purification methods (e.g., deionization, hydrophobicity, etc.).
The color of the filler becomes lighter or turns white	Nickel ions are detached or peeled	Re-hang nickel ions according to the operation of packing regeneration
The filler is brown	The buffer contains reducing agents such as DTT	Reduce the concentration of the reducing agent DTT appropriately, or switch to mercaptoethanol
Protein precipitation occurs during loading	The operating temperature is too low	Load samples at room temperature
	Aggregation of proteins occurs	Add a stabilizer such as 0.1% Triton X-100 or Tween-20 to the sample and all buffers

Notes

1. It is recommended that the buffers and protein solutions used for purification be filtered through a 0.22 μm or 0.45 μm filter before being used on the column.
2. Sterilization: Since the 20% ethanol preservation solution does not have the effect of sterilization and pyrogenization, it is recommended that HyperTrap Ni-NTA Excel Column media can be treated with 70% ethanol for more than 12 h before and during use, or the denickeled 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 research purposes only.

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