HyperChrom Ni-NTA Excel Agarose

Product Description:

HyperChrom Ni-NTA Excel Agarose is an affinity chromatography medium that uses a highly cross-linked agarose gel as a matrix to incorporate a transition metal ion Ni^{2+} with tetracoordination, chemically coupled to a ligand of Nitrilotriacetic acid (NTA). 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 proteins to enable the separation and purification of proteins with and without these amino acids, and with or without these amino acids.

Compared to HyperChrom Ni-NTA FF Agarose (PC2001) and HyperChrom Ni-NTA HP Agarose (PC2002), this product has the following advantages: (i) it has strong binding force with Ni^{2+}, can tolerate 100 mM EDTA, and has better compatibility with buffers, (ii) has high alkali resistance, can withstand 0.5 M NaOH immersion for 48 h, does not need to remove nickel cleaning, avoids cross-contamination, saves time and efficiency.

Product parameters

HyperChrom Ni-NTA Excel Agarose 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 - 3ºC
pH stability *	2-14
Storage	4ºC for 5 years
Transport condition	Room temperature
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 equilibration 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). In order 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. Columns

The following column packing methods are suitable for packing lab-scale chromatography columns:

4.1 Supplies required for column loading.

(1) Chromatography medium: HyperChrom Ni-NTA Excel Agarose

(2) Empty columns: Laboratory-scale empty columns and column-loaders

(3) Required solution:

a) Column solution: 20% ethanol

b) Exhaust solution: 20% ethanol

(4) Column loading tools: sand core funnel, stirring bar, measuring cylinder, etc.

4.2 Preparation before column loading

(1) Calculate the volume of chromatography medium Vm (the volume of the chromatography media part after sufficient sedimentation) required for column loading, and calculate the formula:

Vm = column cross-sectional area X bed height of the column to be mounted X compression ratio of the chromatography medium.

(Note: The compression ratio of HyperChrom Ni-NTA Excel Agarose is 1.15).

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

(3) The appropriate proportion of column suspension for HyperChrom Ni-NTA Excel Agarose chromatography media is 50% to 70%. In order to obtain an accurate volume of chromatography media, the chromatography media can be placed in a graduated cylinder and settled overnight or low-speed centrifugation (3000 rpm, 5 min) can be used to simulate the natural sedimentation effect of the chromatography medium.

(4) Check the empty column to ensure it is clean and leak-free.

4.3 Column loading

(1) 20% ethanol was used to exhaust the bottom filter membrane (screen).

(2) After sufficient exhaust, screw the plug or close the column bottom valve at the column bottom interface, and continue to inject a small amount of 20% ethanol to cover the column bottom.

(3) Adjust the column to vertical.

(4) The column head was connected to the chromatography system, a low flow rate of 5 m L/min

was provided through the chromatography system, and the column head membrane (screen) was vented with 20% ethanol.

(5) Stir well the prepared chromatography media suspension with a stir bar and pour it slowly into the prepared empty column at one time.

Note: If the volume of the suspension exceeds the volume of the empty column, it should be extended by using a column loader or connecting another empty column tube with a connector.

(6) Place the degassed column head into the chromatography column, fit the suspension surface well, and exclude any air bubbles. Then tighten the stigma head seal.

(7) Start the system pump, adjust the flow rate to 300 cm/h, and press the bed using the liquid flow. During the period, the pressure should not exceed 0.3 MPa. In case of overpressure, the flow rate needs to be reduced (see the table below for flow rate conversion).

CID / LFR / V/FR	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
150 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 for different column formats

(8) After the column bed is stable (the rubber surface does not fall again), mark the position of the rubber surface currently. Stop the pump and press down the column head to 2-3 mm below the marked position.

(9) Re-administer the flow rate of 300 cm/h, and if the glue surface does not drop anymore, the column is loaded. If the glue surface drops, repeat steps 8-9.

Note: The recommended workflow speed should not exceed 75% of the column loading flow rate.

5. **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, VR = reserved volume, Wh = 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

6. 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: The loading volume and loading volume of the sample on the HyperChrom Ni-NTA Excel Agarose were determined based on the binding capacity measured in 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.

7. 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.

8. Destruction & Recycling

- Since Ni Chromstar FF 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 that have come into contact with biologically active samples such as viruses and blood, please follow local biosafety requirements before destroying or disposing of them.

9. 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.

10. Frequently asked questions, FAQs.

Issue	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

Precautions

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 20% ethanol preservation solution does not have the effect of sterilization and pyrogenization, it is recommended that HyperChrom Ni-NTA Excel Agarose 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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