BIO-LINK

ChromaX®
MaXtar COLL 400
Multimodal Chromatography Resin

Instructions

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01 Introduction

MaXtar COLL 400 is a multimodal chromatography resin. Different from traditional chromatography resins, MaXtar COLL 400 consists of two layers of different structures. Its outer shell is a porous passivation layer to ensure that macromolecules above 400 kD would not enter the pores but flow directly through the outer water instead. Its core is a spherical inner core coupled with a ligand of both hydrophobic and negative charge adsorption, which helps to maximize the binding of impurities such as host proteins and nucleic acids. It is suitable for the separation and purification of various macromolecular biological samples, such as viruses and virus-like particles.

MaXtar COLL 400 provides superior performance over traditional single-modal chromatography resins:

(1) The unique double-layer structure design helps to process macromolecules above 400 kD in flow-through mode, making process optimization easier and linear scale-up more convenient.

(2) The improved MaXtar matrix has excellent rigidity. This helps to achieve higher process flow velocities under low back pressure, improving process efficiency.

(3) Compared with traditional size exclusion chromatography resins, MaXtar COLL 400 has a larger loading amount, which helps to reduce costs.

02 Resin Characteristics

Table 1. Characteristics of MaXtar COLL 400

Type of chromatography resin	Multimodal
Ligand	A ligand of both hydrophobic and negative charge adsorption
Matrix	Highly cross-linked agarose
Mean particle size	90 μm
Outer exclusion limit	400 kD
Ion capacity	0.06–0.1 mmol Cl^-/mL chromatography resin
Dynamic binding capacity	22 mg ovalbumin/mL chromatography resin*
Recommended flow velocity	90–400 cm/h
Max. flow velocity	700 cm/h
Max. back pressure	0.5 MPa
Temperature stability	4–30 °C

* Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 200 cm/h at 10 cm bed height for 2 mg/mL ovalbumin in 0.02 M Tris-HCl and 0.15 M NaCl solution, pH 7.5

03 Chemical Resistance

Table 2. MaXtar COLL 400 chemical resistance table

pH stability*	3–14
Chemical stability	All commonly used aqueous solutions, 30% isopropanol**, 75% ethanol**, 1 M NaOH, 6 M guanidine hydrochloride, 8 M urea
Intolerable	Oxidizers and citrate buffer

* No significant change was observed at its subsequent chromatographic performance after being stored at 40 °C and pH 3–14 for 7 days

** v/v

04 Column Packing

The following method is suitable for the packing of laboratory-scale chromatography columns. For methods for packing industrial chromatography columns, please consult local technical support.

4.1 Materials

(1) Chromatography resin: MaXtar COLL 400

(2) Column: laboratory-scale empty chromatography column and column packing reservoir

(3) Solvents and detergents:

a. Packing solution: 20% ethanol and 0.2–0.4 M NaCl solution

b. Degassing solution: 20% ethanol

(4) Packing tools: glass filter, stirring rod, and graduated cylinder

4.2 Preparation before packing

(1) Calculate the volume V_m of chromatography resin required for packing (the volume of chromatography resin after adequate sedimentation) according to the following formula:

V_m = column cross-sectional area × packed bed height × compression packing factor

Note: The compression packing factor of MaXtar COLL 400 is 1.15.

(2) Use a glass filter to wash the resin over to the packing solution. Wash 3 times with 2 column volumes (CV) of packing solution. Gently stir with a plastic spatula between additions.

(3) Pour the washed resin from the funnel into a beaker. Add packing solution to obtain the indicated slurry concentration (50%–60%) for the resin. To obtain the exact volume of the resin, place it in a graduated cylinder for overnight sedimentation, or centrifuge it at a lows speed (3000 rpm) for 5 min to simulate the natural sedimentation of the resin before measurement.

(4) Check the empty column to use to ensure that it is clean and free of liquid leakage.

4.3 Packing method

(1) Pump 20% ethanol into the column through the bottom inlet to remove any air trapped under the net.

(2) Apply 20% ethanol 2 cm over the column end piece and close the bottom piece.

(3) Adjust the column to be vertical.

(4) Connect the column head to the chromatography system, and degas the filter membrane (sieve) at the column head with 20% ethanol supplied from the chromatography system at a low flow velocity of 5 mL/min.

(5) Pour the resin slurry into the column in one continuous motion along a glass rod held against the wall of the column. This prevents the introduction of air bubbles into the packed bed. Fill the remainder of the column and the reservoir with distilled water immediately.

Note: If the volume of the gel suspension exceeds that of the empty column, connect another empty column tube with the reservoir or a connector to extend the empty column.

(6) Mount the top piece of the column or lid on the packing reservoir and connect it to the pump.

(7) Open the column outlet and start packing by pumping distilled water through the column according to the flow velocity 600 cm/h. During this time, control the pressure and make sure it does not exceed 0.5 MPa. In case of overpressure, reduce the flow velocity.

Table 3. Flow velocity conversion for columns of different sizes

Column inner diameter / Linear flow velocity / Volume flow velocity (mL/min)	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

(8) When the bed has stabilized, close the bottom outlet and stop the pump. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

(9) Connect the pump, open the bottom outlet and continue packing at 600 cm/h for 5 min. If the gel level does not descend any more, it indicates that the column packing is complete. If the gel level keeps descending, repeat Steps 8–9.

Note: The operational flow velocity/pressure should be < 75% of the packing flow velocity/pressure.

4.4 Packing evaluation

The packing quality can be determined by one of the two test methods shown in Table 4. Use the mobile phase to equilibrate the column until the baseline becomes stable. Load the sample into the column, and then continue washing the column using the mobile phase. After the chromatography peaks return to the baseline, end the run and integrate the chromatography peaks to evaluate column packing quality.

Table 4. Two methods for column efficiency testing

	Acetone method	NaCl method
Sample	1% (v/v) acetone aqueous solution	2 M NaCl aqueous solution
Sample volume	1% of column volume	
Mobile phase	Water	0.2 M NaCl aqueous solution
Flow velocity	30 cm/h	
Detector	UV 280 nm	Conductivity

Calculate N/m (number of plates per meter) and As (peak asymmetry) from the UV curve (or conductivity curve) as follows:

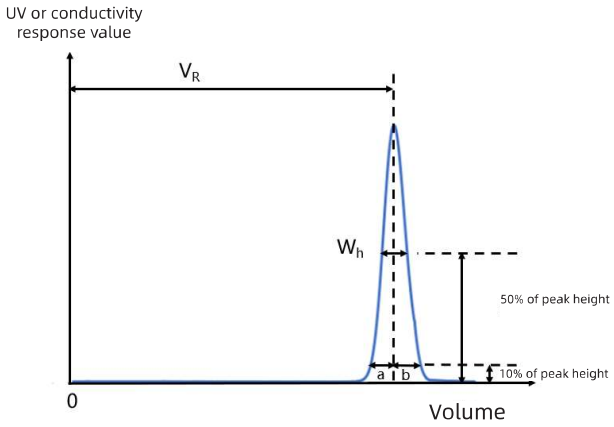
$$N/m = 5.54 \times \left(\frac{V_R}{W_h}\right)^2 \times \frac{1}{L}$$

$$As = b/a$$

A typical acceptable range:

N/m > 3000

0.8 < As < 1.5



L = bed height

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

a = peak width (left half) at 10% of peak height

b = peak width (right half) at 10% of peak height

05 Purification

5.1 Chromatography conditions

(1) **Buffer selection**: The stability of samples in the buffer should be considered. Salt-containing buffers should be used instead of purified water in order to avoid possible non-specific adsorption.

(2) **Flow velocity:** A linear flow velocity of 90–400 cm/h is generally selected according to the bed height of the column.

(3) **Sample pretreatment:** To prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous membrane before being loaded.

5.2 Chromatography steps

(1) **Equilibration:** Use the buffer to fully equilibrate the column until the pH and conductivity are stable and basically the same as those of the equilibration buffer. Generally, this step requires 3–5 CV of equilibration buffer.

(2) **Loading:** Determine the loading volume and loading amount of samples on MaXtar COLL 400 according to the binding capacity measured in the lab-scale.

(3) **Washing and removing impurities:** Use equilibration buffer or other suitable buffer to wash the column until the UV is stable and returns to the baseline.

(4) **Regeneration:** Wash the column with a high-salt buffer (such as 2 M NaCl), or with 1 M NaOH and 30% isopropanol solution.

(5) **Re-equilibration:** Re-equilibrate the column with equilibration buffer.

06 Cleaning-in-place (CIP) and Regeneration

Contaminants, such as lipids, endotoxins, and proteins, may accumulate on the column as the usage count of the resin increases. Regular cleaning-in-place is essential to ensure the column keeps working stably and reliably. The cleaning-in-place frequency can be determined according to the degree of contamination of the chromatography resin. In case of serious contamination, consider performing cleaning-in-place after each use to ensure repeatability of results and maximize the service life of the resin.

For different types of impurities and contaminants, the recommended cleaning conditions are given as follows:

- Removal of strongly binding proteins: Wash the column with 5 CV of 2 M NaCl solution or with a high-salt buffer of a pH not lower than 2, such as 1 M NaAc solution.
- Removal of strongly hydrophobic proteins and precipitated proteins: Wash the column with 5 CV of 1 M NaOH solution, and then with 5–10 CV of purified water to remove the lye.
- Removal of lipoproteins and lipids: Wash the column with 5 CV of 70% ethanol or 30% isopropanol, and then with 5–10 CV of purified water.

Note: 70% ethanol or 30% isopropanol should be degassed before use; a flow velocity of 30–60 cm/h can be selected in cleaning-in-place; reverse cleaning can be used in case of serious clogging.

07 Sanitization

To reduce microbial contamination, it is recommended to use 0.5–1 M NaOH solution to treat the chromatography resin for 15–30 min.

08 Storage

Unopened chromatography resin should be kept in the original container; the packed columns should be soaked in 20% ethanol solution before closing the upper and lower column heads. The storage temperature should be 4–30 ℃ .

09 Destruction and Recycling

Since MaXtar COLL 400 chromatography resin is difficult to degrade in nature, incineration is recommended for disposal of waste chromatography resin in order to protect the environment.

The chromatography resin in contact with biologically active samples such as viruses and blood should be treated properly following local biosafety requirements before destruction or disposal.

10 Ordering Information

Product	Product Code	Quantity
MaXtar COLL 400	1064-1821	25 mL
	1064-1822	100 mL
	1064-1823	500 mL
	1064-1824	1 L
	1064-1825	5 L
	1064-1826	10 L
	1064-1827	20 L

MaXtar COLL 400 is supplied as a suspension in 2% benzyl alcohol with 75% slurry concentration.

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About Bio-Link

Bio-Link is a group of technology-driven businesses that provide process solutions in the life sciences industry. The company focuses on the development and production of the key processing equipment and consumables used in the manufacturing process of recombinant protein drugs, vaccines, antibodies, cell therapies, gene therapies, and other biological products. Bio-Link's portfolio of offerings covers the entire upstream and downstream bioprocess such as cell culture, single-use mixing and storage, chromatography, filtration (ultrafiltration/diafiltration, clarification, and virus removal), and hydration products, as well as process development services. Bio-Link is committed to providing customers with high-quality, innovative products and solutions and strives to build an efficient, safe and competitive biopharmaceutical supply chain eco-system.

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