Sepax Bioscience

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Proteomix POR-S Resin User Manual

1. Product Introduction

Proteomix POR-S ion exchange chromatography media is designed for the separation and purification of biological samples. It uses polystyrene/divinylbenzene (PS/DVB) as the matrix, available in particle sizes of 15 μm and 30 μm, ensuring excellent physical, chemical, and mechanical stability.

The surface of Proteomix POR-S chromatography media is specially treated to enhance hydrophilicity, minimizing non-specific adsorption with biological samples. Through proprietary surface modification technology, spacer arms and ion exchange functional groups are bonded to the hydrophilic matrix, resulting in a strong cation exchange (S) chromatography media. This ensures a high-density and uniform ion exchange surface layer.

Proteomix POR-S ion exchange chromatography media is widely applicable for the separation and purification of biological samples such as insulin, nucleic acids, peptides, heparin, proteins, and more.

Product Characteristics

Rigid matrix can withstand high pressure and high flow rates

High resolution, high column efficiency, and high recovery rate

Wide pH tolerance range

High batch-to-batch reproducibility and easy scalability

Production capacity: > 100 L

2. Safety

For information on the safe use of this product, please refer to the Safety Data Sheet (SDS).

3. Product Properties and Application

3.1. Chromatography Media Chemical Structure and Technical Parameters

Proteomix POR-S is a strong cation exchange chromatography media with a sulfonic acid functional group. The structural schematic is shown in Figure 1, and the specific product technical parameters are provided in Table 1.

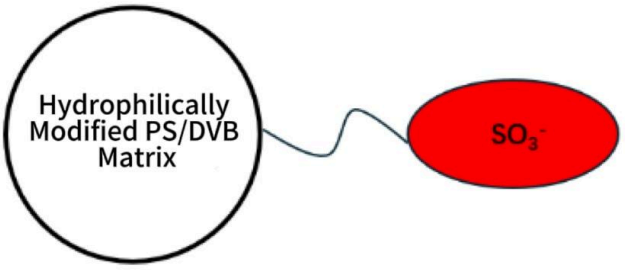

Figure 1. Schematic diagram of Proteomix POR-S chromatography medium ligand structure

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Table 1. Technical Parameters of Proteomix POR-S Chromatography Media

Product Name	Proteomix POR15-S	Proteomix POR30-S
Ion Exchange Type	Strong Cation	
Functional Group	$-SO_3$	
Particle Size	~15 μm	~30 μm
Recommended Flow Rate Range (Or Maximum Operating Flow Rate)*	1800 cm/hr（50 bar）	2000 cm/hr（50 bar）
Dynamic Binding Capacity* (/ml Resin)	≥ 35 mg Lysozyme	≥ 35 mg Lysozyme
Ph Stability (Operation) Ph Stability (Cip)	2 - 13 1 - 14	
Operating Temperature	4 - 35 ℃	
Operating Pressure	≤ 10.0 MPa (100 bar)	
Chemical Stability*	Compatible with buffer salt systems (Tris, phosphate, acetate buffers, etc.), common organic/water systems (acetonitrile, ethanol, etc.)	
Storage Conditions	Please refer to "8. Product Storage" for details	
Transportation Conditions	4-35 ℃, stored in 20% ethanol	
Typical Applications	Insulin, nucleic acids	

*Note:
1. DBC Testing Method: Lysozyme (1 mg/mL) dissolved in 50 mM phosphate buffer (pH = 6.0), linear flow rate of 360 cm/h, detection wavelength of 280 nm, monitoring the value at 10% breakthrough.
2. Maximum Flow Rate Testing Method: Column height of 25 cm, mobile phase is acetonitrile.
3. Chemical Stability Testing Conditions: The resin was soaked in the standard reagents at 40℃ for one week. Results showed that the binding capacity remained above 90% of the original capacity.

3.2. Purification Application

3.2.1. Proteomix POR15-S High-Resolution Purification Application

Chromatography Column Information:
Proteomix POR15-S (4.6 × 250 mm, CV = 9.55 mL)
Detector: UV 280 nm
Sample: Insulin sample
Sample Load: 4 mg/mL

Steps	mobile phase	Residence time min	Rinse Volume CV
Equilibration	Buffer A	6	3
Sample Load	5.93 mL (4 mg/mL)	6	—
Equilibration	Buffer A	6	3
Elution	0-100% Buffer A - Buffer B	6	6
Regeneration	Buffer B	6	3
CIP	0.5 M NaOH	10	3
Equilibration	Buffer A	6	3
Storage	20% ethanol	6	3

3.2.2. **Purification Results for the Insulin Sample**

Proteomix POR15-S demonstrates high resolution. In the purification chromatogram (Figure 2), the target compound is observed in the elution step, significantly increasing the purity of the target product from 53% to 96.9%, while maintaining a high recovery rate of 91.6%.

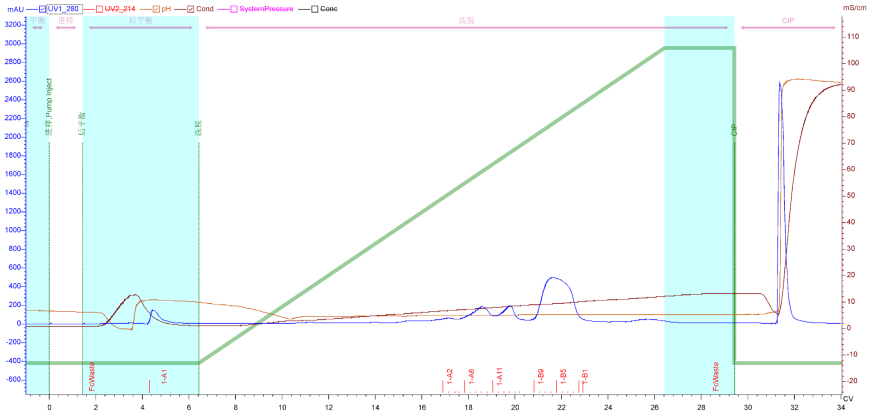

Figure 2: Proteomix POR15-S Purification Chromatogram for Insulin Sample

4. Column installation for chromatography

For the early stages of process development, it is recommended to use pre-packed columns for Proteomix POR15/30-S chromatography media. These can be obtained by contacting sales. For columns with a diameter of 50 mm or larger, it is recommended to use high-pressure columns or DAC for packing. The following column packing methods are provided for reference.

4.1. High-Pressure Column Packing Method

4.1.1. Resin Amount Calculation:

Vslurry = V / P = S × H × F / P

V: Target column volume

P: Slurry ratio

S: Column cross-sectional area

H: Packing height

F: Compression factor

Note: The resin is stored in 20% ethanol upon delivery, with a slurry ratio of 50%. The resin amount is calculated using a 1.20 compression factor.

4.1.2. Displacement of Packing Liquid:

After calculating the resin amount as described above, mix the resin in the packaging container and transfer the required slurry to the displacement container. Use 1 M NaCl solution to perform 2-3 displacements. After displacement, the recommended slurry ratio is 45-65%.

4.1.3. Specific Operation Steps

4.1.3.1. Pour the displaced packing liquid resin into the column tube in one go and quickly install the column head and piping.

4.1.3.2. Compress the resin at a linear flow rate of 100 cm/h, 200 cm/h, 300 cm/h, etc., until the column pressure reaches 1.0 MPa. Maintain the pressure for 20

minutes, adjust the piston to 2-3 mm below the resin surface, and the packing is complete.

4.2. **DAC Packing Method**

4.2.1. Resin Amount Calculation:
Vslurry = V / P = S × H × F / P
V: Target column volume
P: Slurry ratio
S: Column cross-sectional area
H: Packing height
F: Compression factor

Note: The resin is stored in 20% ethanol upon delivery, with a slurry ratio of 50%. The resin amount is calculated using a 1.20 compression factor.

4.2.2. Slurry Preparation:
After calculating the resin amount as described above, mix the resin in the packaging container and transfer the required slurry to the slurry container. Stir for 10-30 minutes to prepare the slurry.

4.2.3. Packing Steps:

4.2.3.1. Install the DAC frit and piping, adjust the oil pressure to the target value (recommended packing pressure is 20-30 bar, and oil pressure should be converted based on the DAC column information).

4.2.3.2. Pour the prepared slurry into the DAC column tube in one go. Lower the piston until the liquid flows out of the upper piping, complete the air evacuation from the upper piping, seal the upper outlet, and open the lower outlet. Lower the piston until it stops, maintain the pressure for 30 minutes, and the packing is complete.

4.2.4. Column Efficiency Standards:

Mobile Phase	0.1-0.5 M Nacl
Sample	1 M Nacl
Injection Volume	1% -2% Column Volume
Flow Rate	60-180 Cm/H
Detection	Conductance
Column Efficiency Standards	Theoretical Number Of Trays ≥ 2000 N/M 0.8≤as≤1.8

4.2.5. Solutions to non-ideal column efficiency
Tail Peak Solution:
1. Reduce slurry concentration
2. Increase packing pressure

Front Peak Solution:
1. Increase slurry concentration
2. Decrease packing pressure

5. Overview of Purification Method Optimization

During the actual purification process, the following common issues may arise. Table 3 provides recommendations for common problems and their solutions. These suggestions can help identify and resolve issues, ensuring smooth method development or production.

Table 3. Common issues in purification and troubleshooting methods

Common Problem	Resolvent
High Back Pressure During Operation	1. Column frit blockage: Clean or replace the frit. 2. Resin pore blockage due to contaminants: Perform CIP operation.
Low Binding Capacity	1. Inappropriate sample pH and conductivity: Recommended sample pH is 7-9, Cond < 6 ms/cm. 2. Poor column packing: Early flow-through occurrence, check column efficiency and symmetry.
Low Recovery Rate	1. Sample overload: Reduce sample load. 2. Inaccurate sample flow rate volume: Calibrate the instrument flow rate. 3. Low elution pH and conductivity: Optimize elution conditions.
CIP Peak Height	Insufficient elution or CIP: Optimize elution conditions and CIP procedure or perform CIP more frequently.

6. Cleaning In Place (CIP)

If impurities are not removed during the regeneration process, leading to column blockage, increased back pressure, or reduced flow rate, online cleaning in the forward or reverse direction can be used to restore column performance. Since online cleaning typically increases the back pressure of the column, it is recommended to use a linear flow rate that is less than 0.5 times of the normal application conditions.

The specific online cleaning method should be selected based on the nature of the impurities.

General Impurities and Routine Cleaning

After each experiment, clean with 0.1-0.5 M NaOH for 3-5 CV.

After NaOH cleaning, it is recommended to rinse with purified water, followed by equilibration buffer to quickly reduce pH.

Cleaning of Hydrophobic Impurities

Wash with 3-5 CV of 70% ethanol or 30% isopropanol.

If further cleaning is required, use 0.5 M NaOH + 30% isopropanol under strong cleaning conditions.

Use a low flow rate during cleaning (approximately half of the working flow rate).

When switching between high-salt buffers and organic solvents, rinse the chromatography column with purified water to prevent precipitation due to salt crystallization.

Cleaning of Other Impurities

For precipitates in the chromatography column, wash with a denaturant such as urea or guanidine hydrochloride, followed by routine CIP cleaning.

7. Sterilization

Since the 20% ethanol or 0.1M NaOh storage solution does not have bactericidal or endotoxin removal effects, it is recommended to treat the chromatography column with 0.5-1.0 M NaOH for 0.5 - 1 hours before and during use to reduce the risk of microbial contamination.

8. Storage Guidelines

The product is sold with 20% ethanol as the storage solution. Upon receiving the resin, please store it under the following conditions:

Unopened resin: Store at 4-35°C in a sealed packaging container, with a shelf life of 60 months.

Used resin:

1) Storage in the chromatography column: After use, flush the column with 3-5 column volumes (CV) of 20% ethanol or 0.1 M NaOH, then store it in a sealed condition at 4-35°C. To prevent ethanol evaporation and microbial growth, it is recommended to replace the storage solution every two months. Since organic solvents, alkalis, and purified water may affect the column material, and prolonged storage in the column may cause the resin bed to dry and crack, long-term storage in the column is not recommended.
2) Storage after column disassembly: Before disassembly, the column should undergo routine regeneration and sterilization procedures. Flush with 3-5 CV of sterile injection-grade water, followed by 3-5 CV of 20% ethanol as the storage solution. Remove the resin and transfer it to a clean and sterilized storage container. Add 20% ethanol storage solution, ensuring the storage solution volume is approximately equal to the resin volume. Store sealed at 4-35°C.

9. Disposal and Recycling

Since the Proteomix POR-S resin is difficult to degrade naturally, it is recommended to dispose of it through incineration or by outsourcing to a third-party disposal service.

10. Ordering Product

Product Name	Type	Particle size	Order number
Proteomix POR15-S	Strong Cation Exchange	15 µm	221115950
Proteomix POR30-S	Strong Cation Exchange	30 µm	221130950

Pre-installed column specifications: 5.0 mL;
Packaging specifications for chromatography media; 1.0 L, 5.0 L, 10 L, 50 L.

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