Sepax Bioscience

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Proteomix POR-Q Resin User Manual

1. Product Introduction

Proteomix POR-Q ion exchange chromatography media is designed for the separation and purification of biological samples. It uses a polymer PS/DVB-based microsphere as the matrix, with particle sizes of 15 μm and 30 μm, offering excellent physical and chemical stability as well as enhanced pressure tolerance.

The surface of the Proteomix POR-Q chromatography media is treated with a special hydrophilic coating developed by Sepax Bioscience, improving hydrophilicity and minimizing non-specific adsorption with biological samples. Through proprietary surface modification techniques, spacer arms and ion exchange functional groups are bonded to the hydrophilic matrix, resulting in a strong anion exchange (Q) chromatography media. This ensures a high density and uniformity of the surface ion exchange layer.

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

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-Q is a strong anion exchange chromatography medium with quaternary ammonium groups as the ligand. The structural schematic is shown in Figure 1, and detailed product technical parameters are listed in Table 1.

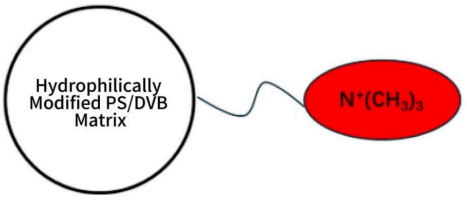

Figure 1. Schematic diagram of Proteomix POR-Q chromatography medium ligand structure

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

Product Name	**Proteomix POR15-Q**	**Proteomix POR30-Q**
Ion Exchange Type	Strong Anion Exchange	
Functional Group	$-^{N+}(CH_3)_3$	
Particle Size (μm)	15.0±2.0	30.0±5.0
Recommended Flow Rate Range (Or Maximum Operating Flow Rate)*	1600 cm/hr（50 bar）	2300 cm/hr（50 bar）
Dynamic Binding Capacity* (/Ml Resin)	≥35 mg BSA	≥25 mg BSA
Ph Stability (Operation) Ph Stability (Cip)	2 - 12 1 - 14	
Operating Temperature	4 - 35 ℃	
Operating Pressure	≤ 10.0 MPa (100 bar)	
Chemical Stability*	Compatible for buffer salt systems (Tris, phosphate, acetate buffer, etc.), conventional organic phase/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 acid	

Note:

1. DBC Testing Method: Bovine serum albumin (BSA, 2 mg/mL) dissolved in 50 mM Tris buffer (pH = 8.5), with a linear flow rate of 180 cm/h, detection wavelength at 280 nm, and monitoring the 10% flow-through value.
2. Maximum Flow Rate Testing Method: Column height of 250 mm, with acetonitrile at 50 bar pressure.
3. Chemical Stability Testing Conditions: The resin was soaked in the standard reagents listed in the table at 40℃ for one week. The results showed that the binding capacity remained above 90% of the original capacity.

3.2. Purification Application

3.2.1. Proteomix POR15-Q High-Resolution Purification Application

3.2.1.1. Purification Experiment for an Oligonucleotide Sample

Chromatography Column Information:
Proteomix POR15-Q (7.8 × 200 mm, CV = 9.55 mL)

Detector: UV 260 nm
Sample: Oligonucleotide sample
Sample Load: 10 mg/mL resin

Steps	**mobile phase**	**Residence time**	**Rinse volume**

		min	CV
Equilibration	20 mM NaOH, pH 12	6	3
Sample Load	10 mg/mL sample solution	6	—
Post-Equilibration	20 mM NaOH, pH 12, 5 CV, collect flow-through	6	5
Wash Impurities	0-10% B (A + 2 M NaCl)	6	5
Elution 2	10-100% B (A + 2 M NaCl)	6	35
Regeneration	100% B	6	5
CIP	0.5 M NaOH	6	5
Equilibration	0.1 M NaOH	6	5

3.2.1.2. Purification results for an oligonucleotide sample

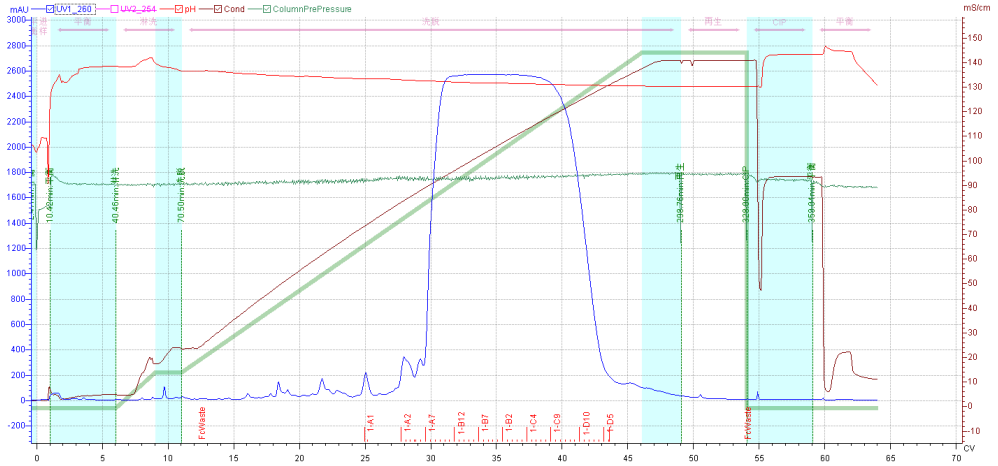

Figure 2. Purification Chromatogram of an Oligonucleotide Sample Using Proteomix POR15-Q

Proteomix POR15-Q offers high resolution, and in the purification chromatogram, the target compound can be observed in the elution step, significantly improving the purity of the target product. The purification results are shown in the table below:

Sample Name	Purity %	Recovery %
Oligonucleotide Sample	85.20	–
Proteomix POR15-Q	96.90	83.57

3.2.2. Proteomix POR30-Q High-Resolution Purification Application

3.2.2.1. Purification Experiment for an Oligonucleotide Sample

Chromatography Column Information:

Proteomix POR30-Q (7.8 × 200 mm, CV = 9.55 mL)

Detector: UV 260 nm

Sample: Oligonucleotide sample
Sample Load: 10 mg/mL

Purification steps	mobile phase	Residence time min	Rinse volume CV
Equilibration	20 mM NaOH, pH 12	6	3
Sample Load	10 mg/mL sample solution	6	—
Post-Equilibration	20 mM NaOH, pH 12, 5 CV, collect flow-through	6	5
Wash Impurities	0-10% B (A + 2 M NaCl)	6	5
Elution 2	10-100% B (A + 2 M NaCl)	6	35
Regeneration	100% B	6	5
CIP	0.5 M NaOH	6	5
Equilibration	0.1 M NaOH	6	5

3.2.2.2. Purification Results for an Oligonucleotide Sample

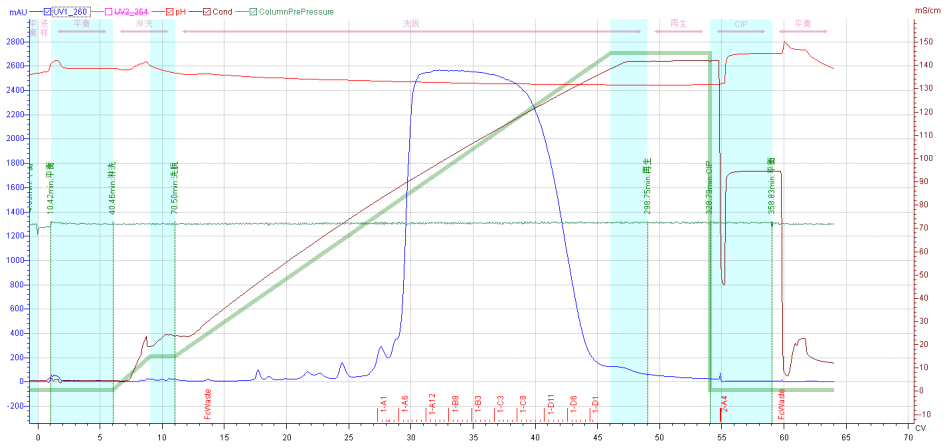

Figure 3. Purification Chromatogram of an Oligonucleotide Sample Using Proteomix POR30-Q

Proteomix POR30-Q offers high resolution, and in the purification chromatogram, the target compound can be observed in the elution step, significantly improving the purity of the target product. The purification results are shown in the table below:

Sample Name	Purity %	Recovery %
Oligonucleotide Sample	85.20	–
Proteomix POR15-Q	95.80	81.57

4. Column installation for chromatography

Chromatography media require different column packing methods for various scenarios. During the early stages of process development, it is recommended to use pre-packed columns, which can be obtained by contacting sales. For columns with an inner diameter of 50 mm and above, it is recommended to use medium-high pressure columns or DAC for packing. The following packing methods can be used as a reference. Column packing is related to pressure-flow rate information, and attention should also be paid to pressure changes during packing.

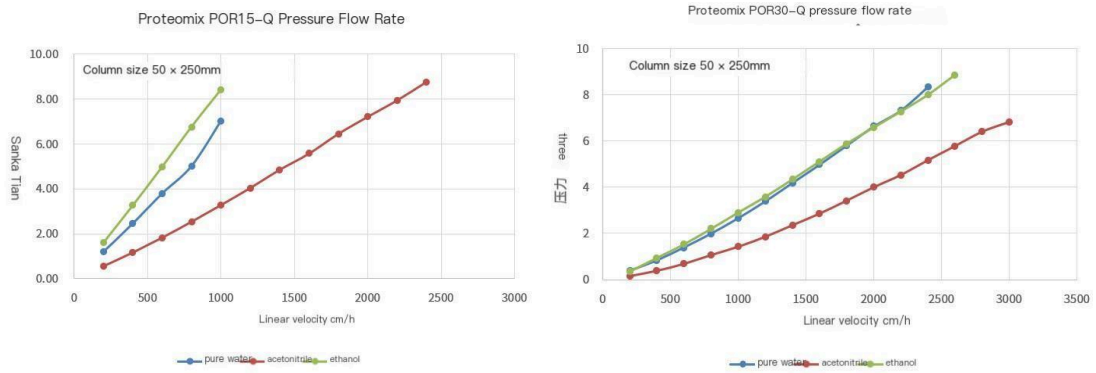

Figure 4. Proteomix POR15/30-Q pressure and flow rate variation chart

4.1. Laboratory scale column packing (ID 6.6 mm - 25 mm)

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

Proteomix POR-Q: 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-Q 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-Q	Strong Anion Exchange	15 µm	221415950
Proteomix POR30-Q	Strong Anion Exchange	30 µm	221430950

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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