Sepax Bioscience

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Proteomix POR50-dT20 Resin User Manual

1. Product Introduction

Sepax Bioscience Proteomix POR50-dT20 Affinity Resin is based on monodisperse PS/DVB polymer beads with a 50 μm particle size, featuring an ultra-large through-pore structure, excellent physicochemical stability, and enhanced pressure resistance. The surface of the Proteomix POR50-dT20 resin is treated with Sepax Bioscience' proprietary hydrophilic coating, providing superior hydrophilicity and minimizing nonspecific adsorption of biological samples. Through a unique surface modification technique, Oligo dT20 molecules are covalently bonded to the hydrophilic matrix, creating an affinity resin specifically designed for the separation of messenger RNA (mRNA).

Most mRNA molecules contain a polyadenylated (PolyA) tail. The Oligo dT20 molecules on the surface of the Proteomix POR50-dT20 resin can form base pairing with the PolyA tail of mRNA, enabling the selective capture of mRNA from IVT transcription products.

Product Characteristics

Purification Process: Simple process, saving development time.
Loading Capacity & Recovery Rate: The ultra-large pore structure provides higher loading capacity for long-chain mRNA, facilitates easier elution, and improves recovery rate.
Impurity Removal: Effectively reduces plasmid DNA and other transcriptional byproducts.
High-Temperature Stability: Suitable for high temperatures (≤65°C) to disrupt unwanted higher-order structures if needed.
Excellent Scalability: Available in pre-packed columns, semi-preparative columns, preparative columns, and bulk resin.
Safety: Made with animal-free raw materials.

2. Safety

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

3. Product Properties and Application

Proteomix POR50-dT20 is an affinity chromatography medium with Oligo dT20 molecules as the ligand. The structural schematic is shown in Figure 1, and the specific product technical parameters are listed in Table 1.

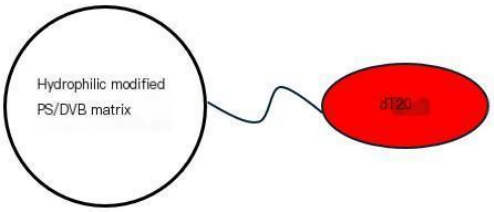

Figure 1. Schematic Diagram of Chromatography Medium Ligand Structure

Table 1. Technical parameters of Proteomix POR50-dT20 chromatography medium

Product Name	Proteomix POR50-dT20
Types of ligands	mRNA affinity
Functional Group	-dT20
Average Particle Size	44.0±6.0
Flow Rate/Pressure Relationship*	800 cm/hr (operating pressure 2 bar)
Dynamic Binding Capacity* (/Ml Resin)	≥ 0.75 mg Oligo A40

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PH Stability (Operation) PH Stability (CIP)	2-12 2-13.7
Working Temperature	2-65 ℃, avoid freezing
Working Pressure	≤ 10.0 MPa (100 bar)
Chemical Stability*	ommon reagents: Water, 0.5 M NaOH, 0.5% SDS, 20 mM EDTA, 5 mM dTT, 2 M MgCl2, and other conventional reagents; Avoid using reagents: Strong oxidizers (e.g., hypochlorite), oxidizing acids (e.g., nitric acid), strong reductants (e.g., sulfites), acetone, tetrahydrofuran, or benzyl alcohol.
Storage Conditions	Please refer to "8. Product Storage" for details
Transportation conditions	4-35 ℃, stored in 20% ethanol
Typical Applications	Capture and purification of mRNA samples with PolyA tail end

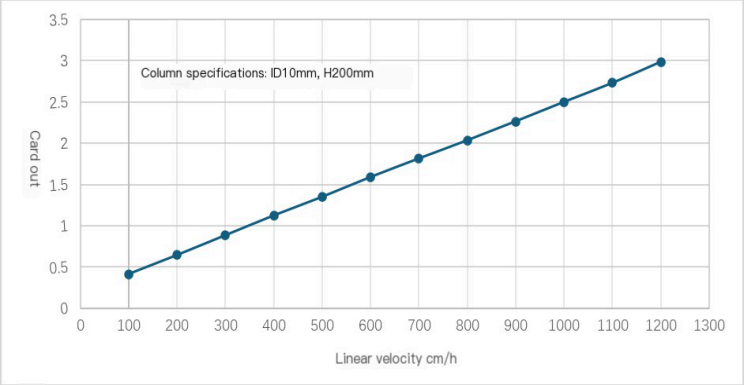
*Note: 1. DBC testing method: residence time of 2 minutes, sample solution is 100 mM Tris+500 mM NaCl containing 0.0333 mg/mL Oligo A40, pH 7.5;

2. Flow rate testing method: under the conditions of column height of 200 mm and column pressure of 2 bar;

3. Chemical stability testing conditions: The fillers were soaked in conventional reagents at 40 ℃ for one week in the table, and the results showed that the loading was over 90% of the original loading.

4. Column Packing Recommendation

Chromatography resin requires different column packing methods for various scenarios. The packing methods for laboratory-scale columns differ significantly from those used in large-scale production. Column packing is related to the pressure-flow rate relationship. Figure 2 shows the pressure-flow rate curve of Proteomix POR50-dT20.



(Column: 10◇200 mm; Mobile Phase: 0.1 M NaCl)

Figure 2. Proteomix POR50-dT20 pressure flow rate curve

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

4.1.1. Preparation

4.1.1.1. Packing equipment: Ensure the FPLC system (e.g. AKTA Pure25) functions normally, especially the pressure detection module and conductivity detection module.

4.1.1.2. Solution preparation: Prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.1.2. Replace storage solvent

Proteomix POR50-dT20 resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with 0.1M NaCl. Quick Replacement Method: Mix the resin thoroughly, take the required amount of slurry, and pour it into the column tube. Install the column head and flush with 0.1M NaCl for 5 CV. Then, transfer the resin into a wide-mouth bottle and add 0.1M NaCl until the resin volume ratio reaches 50-70%. Alternatively, the storage solvent can be replaced using a sedimentation method.

4.1.3. Calculation of slurry ratio

Quick Calculation Method: Mix the slurry thoroughly until the mixture is homogenous, take 20 mL, and add it into a glass column such as a Generik FPLC 10×400 mm glass column. Open the bottom stopper to allow water to drain out until the resin settles and the bed height is stable. Measure the bed height using a ruler and calculate the resin volume. For example, if the bed height is 14 cm, the resin volume is calculated as 14×0.7854=10.9956mL, resulting in a slurry ratio of 54.98%. Alternatively, the slurry ratio can be calculated using an overnight sedimentation method in a graduated cylinder with a resin settling time of 14–16 hours.

4.1.4. Calculation of slurry volume

$V_{slurry} = V / P = S \times H \times F / P$

V_{slurry}: Required slurry volume
V: Target column volume
P: Slurry ratio
H: Target column height
S: Cross sectional area of column
F: Compression factor

For example, if the cross-sectional area of a column with an inner diameter of 10mm is 0.7854 cm^2 and the target bed height for column is 20 cm, the required volume of resin is:

V_{slurry} = 0.7854 cm^2 x 20 cm x 1.15/54.98% = 32.9 mL

*Note: At the laboratory scale and under saltwater packing conditions, the packing material quantity is calculated using a 1.15 compression ratio.

4.1.5. Packing steps

4.1.5.1. Use a pipette to add 32.9mL of slurry to a Generik FPLC 10 x 250mm AF chromatography column tube (using a column mounting ring).

4.1.5.2. Turn on the flow at 100 cm/h and tighten the upper column head onto the column tube. Compress the packing at linear flow rates of 100 cm/h, 200 cm/h, 300 cm/h, maintaining each flow rate for 3.0 minutes. Once the column pressure reaches 3.0 bar, hold for 15 minutes. Then, shut down the chromatography system and lower the column head to the target height using a compression coefficient of 1.05 (based on the marked column bed height). Column packing is now complete.

4.2. Pilot scale column packing (ID 100 mm - 300 mm)

4.2.1. Preparation

4.2.1.1. Location: Clean and dust-free, temperature of 18℃ -35℃, humidity of 45% -65%.

4.2.1.2. Packing equipment and pipelines: Wash the FPLC system and pipelines thoroughly, connect all pipelines, and check whether there is any leakage in the pipelines. If necessary, perform leak tests and verify that all parameters including pressure are displayed correctly.

4.2.1.3. Air Removal: Clean the column thoroughly and remove any bubbles from the upper and lower frits for later use.

4.2.1.4. QC testing equipment: Low pressure chromatography system.

4.2.1.5. Solutions: prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.2.2. Replace storage solvent

Proteomix POR50-dT20 resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with 0.1 M NaCl. For manually packing a column with an inner diameter ranging from 100 mm to 300 mm, the exchange method is as follows: Mix the resin thoroughly, take the required amount of slurry, and pour it into the column tube. Open the outlet valve to allow the 20% ethanol to drain out and replace it three times with 0.1 M NaCl.

4.2.3. Calculation of slurry volume

$V_{slurry} = V/50\% = S \times H \times F/50\%$

V_{slurry}: Required Slurry Amount
V: Target column volume
H: Target bed height
S: Cross sectional area of column
F: Compression factor
For example, if the cross-sectional area of a column with an inner diameter of 200mm is 314cm^2 and the target height for column is 20 cm, the required volume of slurry is
V_{slurry} = 314 cm^2 x 20 cm x 1.20/50% = 15.1 L
*Note: Compression factor of 1.20 is applied, resin is stored in 20% ethanol.

4.2.4. Packing method

4.2.4.1. As described in the process for replacing the storage solvent, resuspend the resin thoroughly, close the bottom column valve, and allow the resin to settle naturally. Once the resin bed surface has settled more than 5 cm below the column top, install the column head and make sure there are no air bubbles. Tighten the column sealing ring and open the bottom column valve. Using 0.1M NaCl as the mobile phase, start the pump and set the flow rate to 100 cm/h to accelerate the resin settling. Continue to flow until the bed height stabilizes and then mark the final bed height.

4.2.4.2. Turn off the chromatography system and wait for the column pressure to drop to zero before closing the bottom valve. Rotate the four-way valve on the column head to the liquid discharge pipe, then lower the column head to the target bed height using a compression factor of 1.13 (based on the marked column bed height).

4.3. Production scale column packing (ID 300 mm -1200 mm production column installation)

4.3.1. Preparation

4.3.1.1. Location: Clean and dust-free, temperature of 18℃-35℃, humidity of 45%-65%.

4.3.1.2. Packing equipment and pipelines: Wash the FPLC system and pipelines thoroughly, connect all pipelines, and check whether there is any leakage in the pipelines. If necessary, perform leak tests and verify that all parameters including pressure are displayed correctly.

4.3.1.3. Air Removal: Clean the column thoroughly and remove any bubbles from the upper and lower frits for later use.

4.3.1.4. QC testing equipment: Low pressure chromatography system.

4.3.1.5. Solutions: prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.3.2. Replace storage solvent

Proteomix POR50-dT20 resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with 0.1M NaCl. Calculate the required amount of slurry using the method below, pour it into the slurry tank, and allow it to settle. After settling, decant the supernatant, add an equal volume of water, mix thoroughly, and allow it to settle again. Repeat this replacement process three times.

4.3.3. Calculation of slurry volume:

Vslurry = V/50% = S×H×F/50%
Vslurry: Required slurry volume
V: Target column volume
H: Target column height
S: Cross sectional area of column
F: Compression factor
For example, if the cross-sectional area of a column with an inner diameter of 600 mm is 2826 cm2 and the target bed height for column is 20 cm, the required volume of slurry is

Vslurry=2826 cm2 x 20 cm x 1.20/50% ≈ 135.6 L.

4.3.4. Packing steps

Eliminate air bubbles in the resin pipeline.

Start the pump and set the linear velocity to 300 cm/h to draw the required amount of resin.

After resin transfer is complete, flush the resin remaining in the pipeline with water.

Column Compression: Lower the column head at a linear velocity of 60 cm/h to compress the resin. When the column head is 2–3 cm above the resin surface, record the resin bed height and compress the column bed using a compression factor of 1.14.

4.4. Reference standards for column efficiency testing and evaluation

After packing the Proteomix POR50-dT20 column, the column efficiency testing method and evaluation standards can be referred to in Table 2.

Sample	1.0 M NaCl
Sample Volume	1.0-2.0% CV
Mobile Phase	0.1-0.5 M NaCl
Flow Rate	60-180 cm/h
Detector	Cond.
Qualified Standards	Asymmetry: 0.8-1.8; Theoretical plate number: ≥ 2000/m

Table 2. Column Efficiency Testing Methods and Evaluation Standards

4.5. Solutions to non-ideal column efficiency

4.5.1. When a tailing peak occurs, solutions include:

Reducing slurry concentration: Decreasing the overall proportion of packing material.

Increasing packing flow rate: Raising the maximum column packing pressure.

4.5.2. When a fronting peak occurs, the solutions are the opposite of those for tailing peaks.

4.5.3. For low column efficiency: Repacking the chromatography column and reducing the testing flow rate.

4.5.4. For peak splitting: Cleaning or replacing the frit and checking the test sample.

4.5.5. For column cracking: Increasing packing pressure during column packing, ensuring the mobile phase is degassed, and fully removing bubbles when connecting the column head.

5. Introduction to Purification Method Optimization

Proteomix POR50-dT20 is used for the capture (purification) of poly A tail mRNA samples. When developing the method, the optimization strategy in Table 3 can be referred to improve the purification effect, which helps to accelerate process development.

Table 3. Proposed Optimization Strategies for Purification Process of Proteomix POR50-dT20

step	mobile phase	Optimization direction
Equilibration	10 mM Tris, 5 mM EDTA, 0.5-1.0 M NaCl, pH7.0	Adjust salt concentration based on the balance between loading and purity while avoiding sample aggregation
Sample Loading		Factors affecting load capacity: 1) Molecular Influence: Molecular size and length of the PolyA tail; 2) Salt Concentration: Loading increases as salt concentration increases, as long as sample stability allows; 3) Residence Time: Recommended time: 5-6 minutes; 4) Sample Concentration: Generally, the higher the concentration

		under the same conditions, the higher the loading.
Post-Equilibration	10 mM Tris, 5 mM EDTA, 0.5-1.0 M NaCl, pH7.0	
Washing	10 mM Tris, 5 mM EDTA, 0.25 -0.5 M NaCl, pH7.0	1) Adjust the salt concentration based on binding strength to wash away some impurities without affecting yield; 2) For the first screening of washing conditions, try a gradient from low to high salt concentration.
Elution	H2O	1) Adjust elution volume based on actual conditions; 2) If elution is difficult or the peak tails, adjust the pH of the mobile phase to mildly alkaline (pH 7.5-8.0) using buffer salts; 3) If the elution peak splits, add 5-10 mM Tris to adjust the charge of the sample and ensure uniform elution.
CIP	0.1 M NaOH	
Cleaning	H2O	
Storage	20% ethanol	Ethanol causes high backpressure, so flow speed can be reduced accordingly.

6. Cleaning In Place (CIP)

If impurities cannot be removed through 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 generally increases column back pressure, it is recommended to monitor column pressure in real time to ensure it remains within the pressure tolerance of both the chromatography system and the resin.

The specific online cleaning method should be determined based on the nature of the impurities:

General impurities and routine cleaning:

Wash with 5 column volumes of 0.1–0.2 M NaOH. For more strongly adsorbed impurities, short-term washing with 0.3–0.5 M NaOH can be used.

Strongly hydrophobic impurities:

Wash with 3–5 column volumes of 70% ethanol or 30% isopropanol.

7. Sterilization

Since 20% ethanol or 10 mM NaOH storage solutions do not have sterilization or endotoxin removal effects, it is recommended to treat Proteomix POR50-dT20 with 0.5 M NaOH for 0.5 to 1.0 hours before and during use to reduce the risk of microbial contamination.

8. Storage Guidelines

Proteomix POR50-dT20 resin is sold in a storage solution of 20% ethanol or 2% benzyl alcohol. Upon receiving the resin, please store it under the following conditions:

Unopened resin: Can be stored long-term at 2–8°C. The shelf life is 60 months.

Used Resin:

1) Chromatography column storage: Store at 4–35°C. After washing with 3–5 column volumes (CV) of 20% ethanol or 10 mM NaOH, seal the column for storage. 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 may cause the column bed to dry and crack, long-term storage in the chromatography column is not recommended.

2) Resin storage after column disassembly: Before disassembly, the chromatography column should undergo routine regeneration and sterilization procedures. Wash with 3–5 CV of sterile injection-grade water, followed by 3–5 CV of 20% ethanol as a storage solution. Transfer the resin into a clean, disinfected storage container, add 20% ethanol, ensuring the storage solution volume is approximately equal to the resin volume, and store it sealed at 2–8°C.

9. Disposal and Recycling

Since the Proteomix POR50-dT20 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	Product Number
Proteomix POR50-dT20	Affinity	65 µm	270965990

Prepacked column specifications: 5.0 mL; Packaging specifications: 1 L, 5 L, 10 L, 50 L

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