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Proteomix POR50-dT20 Affinity Resin User Manual

Product Description

Sepax Proteomix POR50-dT20 Affinity Resin uses 50 μm monodispersed PS/DVB particle as the matrix. The resin has a perfusive porous structure with large through pores and has good physical and chemical stability and good pressure resistance. Oligo dT20-mer is then conjugated to bead surface through a proprietary method. The resulting affinity resin is specially designed and highly optimized for the isolation of messenger RNA (mRNA).

Most mRNA molecules contain a tail of poly(adenylic acid) (polyA tail), up to 250 bases in length. The Monomix dT20 Affinity Resin surface allows capture of mRNA though base pairing between oligo dT20-mer and the mRNA polyA tail using a simple and convenient chromatography procedure.

Technical Specifications

Resin Type	Proteomix POR50-dT20
Base Matrix	Hydrophilic PS/DVB
Particle Size D_{50}	50 μm
Average Pore Size	1000 Å
Functional Group	Oligo dT20-mer
dT20 ligand density	≥2.0 mg/mL resin
DBC Based on Oligo A40	≥0.75 mg/mL resin
Max Linear Flow Rate	1000 cm/hr
Operating Temperature	4-65 °C
pH Stability	2-12
Operating Pressure (Process FPLC)	≤10 bar (1 MPa)
Operating Pressure (Analytical HPLC)	≤100 bar (10 MPa)
Mobile Phase Compatibility	Compatible with common salt buffers, organic/aqueous solutions (acetonitrile, ethanol, etc.), and common additives for mRNA purification
Long-term Storage	Store in 20% ethanol aqueous solution, 2-8℃. Do not freeze resin or column.
CIP	0.1-0.5M NaOH. Recommend starting with 0.1M NaOH to prolong resin life

* mRNA binding ability test is based on a 1000 nt mRNA sample, binding conditions 10 mM Tris-HCl, 1 mM EDTA, 5 mM DTT, 1.0 M NaCl, pH 7.5.

Characteristics

- Provides efficient capture and release under standard mRNA purification conditions, simplifies subsequent purification steps, and maximizes overall production efficiency.
- Large pore sizes which enables high loading capacity for large mRNAs, easier elution, and high recovery rate.
- Decreases process development time and enhances productivity.
- Allows reduction in plasmid DNA and other transcription mix components.
- Stable at elevated temperatures for the breakdown of undesired higher order structures, if needed.
- Excellent scalability. Available as prepacked columns, semi-prep columns, prep columns, and bulk resin.
- Non-animal derived

Instructions for Use

1. Safety Precautions

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

2. Resin Preparation before Use

This product is normally shipped in an aqueous solution containing 20% ethanol and should be washed with deionized water before getting in contact with salt buffer solutions. This can be accomplished by rinsing with 3 times column volume of deionized water and can be part of the column packing procedure (3.3)

3. Column Packing

3.1 Calculate column volume (CV): CV = column cross-sectional area (πr^2) x bed height (h), r is the column radius;

3.2 Gently stir the resin to completely disperse it to form a uniform slurry. Measure the required volume of the resin slurry and pour it into a clean transparent glass or plastic vessel. After natural sedimentation, decant the supernatant, add 3 CV of deionized water, gently stir evenly and then settle for about 30 minutes, decant the supernatant, and repeat 3 times;

3.3 After removing the supernatant, add column packing buffer (1M NaCl solution) to make 60-70% resin slurry (volume based), stir evenly and soak the resin for more than 12 hours (overnight);

3.4 Measure about 1.05 times CV of the resin prepared from previous steps and gently stir evenly to make the final packing slurry. Pour it into a column with filter plate with proper pore sizes relative to the resin particle sizes at the bottom to allow packing buffer to flow through and resin to settle steadily;

3.5 Place a flow distributor on the top of the column, press down the resin bed and connect to a pump;

3.6 Use 2-3 CV column packing buffer to flush the resin bed, compress resin beds at 2 times of normal working flow rate. The distributor position may be adjusted during the compressing process to ensure tightness of the resin bed. It is not recommended to use suction or gravity-only sedimentation to pack a column, especially for columns with a bed height of more than 10 cm;

3.7 QC Testing Method: Evaluation of column packing quality is carried out using a low molecular weight or unretained compound. The specific operating parameters are as follows:

Sample	2 M NaCl
Sample Volume	1.0–2.0% of bed volume
Mobile Phase	0.5 M NaCl Solution
Flow Rate	100 cm/hr
Detector	Conductivity
Specifications	Tailing Factor: 0.8-1.5 Plate Count: ≥ 2000/m

3.8 In case of non-ideal result, such as peak tailing, solutions include:

- Reduce the concentration of slurry
- Increase packing flow rate
- Extend packing time

If peak fronting occurs, solutions are the opposite of the above

4. Column Use

4.1 Select and optimize a buffer system according to specific characteristics of targeted molecules to be separated/purified or analyzed. A common example is Equilibration buffer: 10mM Tris, 1mM EDTA, 5mM DTT, 1.2M NaCl, pH 7.4; Sample buffer: 10mM Tris, 1mM EDTA, 0.5%SDS, 5mM DTT, pH 7.4;

4.2 Equilibrate the column with about 3-10 CV of equilibration buffer until conductivity and pH of effluent are constant;

4.3 Sample Loading: The sample loading volume should be determined according to resin loading capacity and purity of targeted molecule in crude sample;

4.4 Post equilibration and Washing: After loading is completed, continue to pump 3-10 CV of equilibration buffer until a stable baseline is obtained;

4.5 Elution: Flow 3-10 CV of a low salt buffer or deionized water, collect the target mRNA;

4.6 CIP: Flush the column with 5 CV of 0.1 M NaOH at ambient temperature; then flush the column with 10 CV of equilibration buffer to reach baseline equilibrium;

4.7 Column Storage: After use, rinse the column with 10 CV of water and 20% ethanol solution, and store the column at 2-8°C.

Note: To ensure long lifetime of the column, all samples and buffers must be filtered through a 0.45 μm membrane before use.

Storage

Chromatographic resins that will not be immediately used is recommended to store in an aqueous solution containing 20% ethanol at 2 to 8°C in a sealed container. Do not freeze resin or column.

Order Information

Product	Pack	Part Number
Proteomix POR50-dT20	Resin*	2221509D0
	1 mL Cartridge	2221509D0-70025
	4.2 mL Cartridge	2221509D0-750100
	5 mL Cartridge	2221509D0-160025
	4.6 x 50 mm	2221509D0-4605

*Resin pack size available in 50, 100, 250, 500 mL & 1, 5, 10, 50L