thermo scientific | PRODUCT INFORMATION SHEET

POROS™ Oligo (dT)25 Affinity Resin

Pub. No. 100092591 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product information

Product description

The POROS™ Oligo (dT)25 Affinity Resin is a rigid, 50 µm polymeric resin that is designed for the isolation of messenger RNA (mRNA). The resin backbone consists of crosslinked poly(styrene divinylbenzene).

A polyhydroxyl surface coating provides low non-specific binding. The surface is functionalized with poly(dT) and allows capture of mRNA though base pairing with the mRNA polyA tail.

The POROS™ Oligo (dT)25 Affinity Resin provides efficient capture and release under standard mRNA purification conditions. It thereby decreases process development time and enhances productivity. In addition, the selective nature of this resin allows reduction in plasmid DNA and other transcription mix components.The resin is also stable at elevated temperatures for the breakdown of undesired higher order structures, if required.

Storage

Store resins at 2°C–8°C. Do not freeze.

Specifications

Table 1 POROS™ Oligo (dT)25 Affinity Resin product characteristics

Characteristic	Description
Support matrix	Cross-linked poly(styrene-divinylbenzene)
Surface functionality	dT-25mer
Ligand density	0.3 µmol dT/mL resin
Shipping solvent	18% ethanol
Average particle size	50 µm
Mechanical resistance	100 bar (1,450 psi, 10 MPa)
Suggested compression factor	1.06
Operating temperature	2 to 65°C Do not freeze

Table 2 POROS™ Oligo (dT)25 Affinity Resin chemical and thermal resistance

Characteristic	Description
pH Range	2–13
Ionic strength range	0 to 5 M, all common salts
Buffer additives	Common agents for mRNA purification, including 0.5 M NaOH, 2 MMgCl$_2$, 20 mM EDTA. Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), strong reducing agents (such as sulfite), acetone, THF, or benzyl alcohol.
Solvents	Water, 0 to 100% alcohol, acetonitrile, 2 M acetic acid, 1 M HCl, other common organic solvents Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), strong reducing agents (such as sulfite), acetone, benzyl alcohol, or THF.
Flow rate	Adjust flow rate depending on performance. Do not exceed upper pressure limitations.

Performance characteristics

The pressure-flow curve of POROS™ Oligo (dT)25 Affinity Resin is shown in Figure 1. POROS™ resins can be operated at high linear flow rates with a pressure drop that allows for use with conventional low-pressure chromatography columns and systems. Backpressure is affected by buffer composition, viscosity, and temperature.

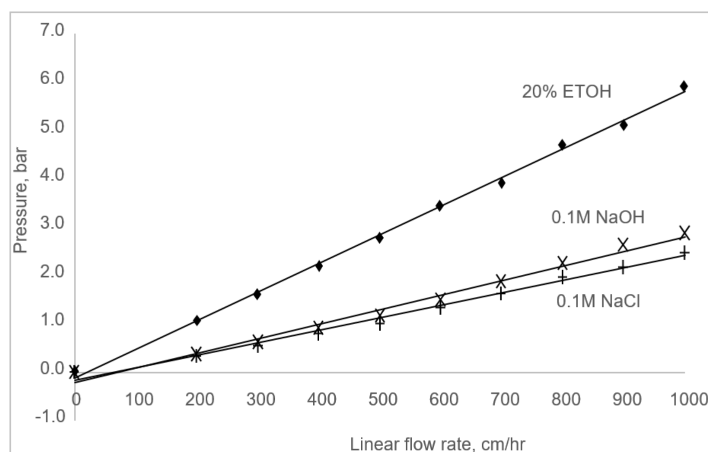

Figure 1 Pressure-flow properties for POROS™ Oligo (dT)25 Affinity Resin (bed dimensions: 1.5 cmD × 10 cmL, compression factor: 1.06, column packing: 0.1M NaCl)

Product usage: See "Product use" on page 7

Pack and qualify the column

Packing guidelines

- Resins are supplied as approximately 50–55% slurry in 18–20% ethanol. For column packing, exchange the shipping solution with 0.1 M sodium chloride or the desired packing solution.
- Resins are mechanically rigid and can be packed effectively in low-pressure glass columns and in high-pressure stainless steel columns. Columns can be packed with traditional flow pack, axial compression, or pack-in-place/stall pack packing methods.
- A compression factor of 1.06–1.5 is recommended for column packing. Compression factor is defined as the ratio of gravity-settled volume to packed-bed volume. This factor, along with the slurry concentration, is used to determine the volume of slurry required to yield the intended final column volume (CV).
- Standard 10–23 µm screens (frits) can be used.
- For best results, use a column tube or column fitted with an extender large enough to contain the entire slurry so that the bed can be packed all at once. Funnel-like column packing devices do not work well for packing POROS™ resins.

Prepare slurry: lab-scale columns (≤100 mL)

Buffer-exchange using a 0.2–0.45 µm bottle-top filter or sintered-glass filter:

1. Transfer the required volume of resin slurry to the top of a bottle-top filter.
2. Apply vacuum to remove the shipping solution.
3. Resuspend the resin with the desired packing solution to a slurry density of approximately 50%.
4. To remove the storage solution, repeat the vacuum and resuspension steps for at least 3 times.
5. Resuspend the resin with the desired packing solution to a slurry density of approximately 50%.
6. Verify that the slurry density is 50–70% by sampling 10–100 mL of slurry and allowing it to gravity-settle in a graduated cylinder for at least 4 hours.
7. If needed, adjust the slurry concentration to 50–70%.

Prepare slurry: lab scale and larger scale columns (>100 mL)

Buffer-exchange using repeated gravity settling:

1. Allow the resin to settle in the shipping container. Settling requires >4 hours because the density of the resin is approximately that of water.

   As vessel height increases, settling can require more time. Large vessels may need to settle overnight to ensure good separation. As vessel size increases, the supernatant can be pumped off.

2. Being careful not disturb the bed, decant the supernatant.

   Some particles/turbidity may be present in the decanted supernatant because beads can pull off the settled bed or come loose from the carboy side walls. This is not problematic.

3. Replace the supernatant with the same volume of the desired packing solution.
4. Resuspend the resin by gentle agitation by hand, resin wand, air sparging, paddle, flat bed shaker, top-mounted impeller mixer, or rotary mixer, then allow the resin to settle by gravity.

   As with any resin, do not use a magnetic stirrer. It can abrade the particles and cause fines to form.

5. Repeat steps 1 to 4 two to three times to thoroughly exchange into the packing solution.
6. Verify that the slurry concentration is 50–70% by sampling 10–100 mL of slurry in a 10–100 mL graduated cylinder (respectively) and gravity settling for >4 hours.
7. If needed, adjust the slurry concentration to 50–70%.

Pack the column

For larger columns, use a 3- or 4-way valve on the top and bottom of the column (if possible) to allow bypass of the column and avoid introducing air during packing and column use. Place a calibrated pressure gauge at the inlet of the column.

When you adjust the flow rate to form the bed, you may observe some turbidity in the eluent as packing starts. Turbidity will clear as packing proceeds and 1–2 bed volumes of packing buffer pass through the column.

1. Determine the required slurry volume:

   Required slurry volume = target column volume (CV) / slurry ratio x compression factor (CF)

   Example for a 40 cmD × 20 cmL, 25-L column using slurry with a 56% slurry ratio:

   25 L / 0.56 × 1.06 = 47.3 L slurry required

2. Ensure that the column outlet is closed and plumbed directly to waste. Do not connect the column outlet to the chromatography system. Plumbing into the system creates backpressure that fights against the inlet pressure trying to settle the bed and pack the column.

3. Ensure that the column is level and locked in place before starting the pack.

4. Deliver the required slurry volume to the column by hand or with a diaphragm pump, as dictated by your equipment and the intended packing procedure. Use a squirt bottle containing packing solution to remove any residual resin from the column wall.

   POROS™ resin beads have a skeletal density similar to the density of water and do not settle rapidly. Do not allow the resin to gravity-settle in the column before packing.

5. With the column inlet line connected to the system and the bottom outlet closed, bring the primed top flow adapter to 1–2 cm from the slurry level, then tighten the O-ring. Do not push the resin up and over the O-ring. Change the top valve to force the air and liquid out the top of the adapter and to waste using the bypass line. Continue to lower the adapter slowly to remove the bubbles from the top of the column. Do not allow large air bubbles between the top adaptor and the top of the resin slurry.

6. Change the valve back to flow through the system on the top, then open the column bottom.

7. Increase the flow rate to the maximum or desired flow rate and pressure obtainable with the equipment used:
   - **Flow packing** – Pack at a flow rate at least 50% greater than the maximum operating flow rate for your chromatography operation, with an approximate final packing pressure drop of 3 bar. This flow should yield a pressure higher than the desired operating pressure for all column steps. For smaller diameter columns (≤1 cm), we recommend higher packing flow rates of 1,000–2,000 cm/hour.
   - **Flow packing with axial compression** – Place the top flow adaptor at a height that will accommodate all of the slurry. Pump the slurry into the column using the slurry nozzle and follow with 0.1 M sodium chloride to chase the remaining resin or use extra slurry to avoid introducing air into the line.
     Pack at flow rates/pressures up to the limits of the column. Pack at a flow rate at least 50% greater than the maximum operating flow rate for your chromatography operation. This flow should yield a pressure higher than the desired operating pressure for all column steps.
     After about 2 CVs, lower the top adapter to the target bed height based on the compression factor and slurry concentration calculations.
   - **Axial compression** – Pack at flow rates/pressures up to the limits of the hydraulics of the column (at least 2.5 bar). Add the slurry to the column as you would for flow packing, but proceed directly with axial compression by lowering the adapter to the target bed height based on the compression factor and slurry concentration calculations.
   - **Pack-in-place/Stall pack** – Pack at flow rates/pressures up to the limits of the column. Lock the top adapter into place at the desired bed height. Set a packing pressure of 3 bar on the slurry transfer skid and pump resin into the column until all of the required resin has been transferred or the pump stalls.

8. **Flow packing only**: Continue flow until a clear space forms between the column top adjuster and the slurry (~2 CVs). Monitor the pressure; it will gradually rise as the column packs.

9. After the bed is formed, bring the adapter down by closing the column outlet and displacing liquid through the top of the adapter to waste through the bypass line. Lower the adapter to the target bed height based on the compression factor and slurry concentration calculations.

10. After the column is packed, flow 2–3 CVs of packing solution through the packed bed at the operating flow rate to stabilize the bed.

    The flow rate used should generate no more than 80% of the final packing pressure.

11. If you will reverse the flow of the column during operation, condition the column in upflow:
    - Flow 2–3 CVs in upflow at the operating flow rate.
    - Flow 2–3 CVs in downflow at the operating flow rate, then adjust the adapter if needed.
    - Flow 2 CVs after you adjust the adapter.

Qualify the column

To qualify the integrity of a packed column, determine HETP (height equivalent to a theoretical plate) and asymmetry using a non-binding analyte (a “plug”). Degas solutions before use to avoid gassing out during operation.

Recommended column qualification conditions

Condition	Recommendation
Flow rate	Target operating flow rate (cm/hour)
Equilibration buffer	0.1 M sodium chloride
Plug solution	1 M sodium chloride
Plug volume	2% of column volume

Guidelines

- Ensure uniform column plumbing:
  - Avoid using reducers to connect different tubing sizes.
  - Minimize and keep consistent the column tubing lengths between the plug solution to the column inlet and the column outlet to the detector(s).
- Execute at the flow rate that is defined for the intended unit operation, typically 100–300 cm/hour.
- Equilibrate with at least 4 CVs of equilibration buffer before injection.

Setting specifications

Qualification results depend on several factors, including the:

- Solutions and method used
- Scale
- Column hardware
- Chromatography system

After you define a column qualification procedure for a specific system (column plus chromatography system), base the qualification acceptance criteria on historical values and ranges instead of theoretical qualification results. Performing the column qualification method consistently and reproducibly is critical to obtaining meaningful results.

Qualification example

Figure 2 shows a typical column qualification peak. The peak void volume of a POROS™ column is typically 0.7–0.8 CV.

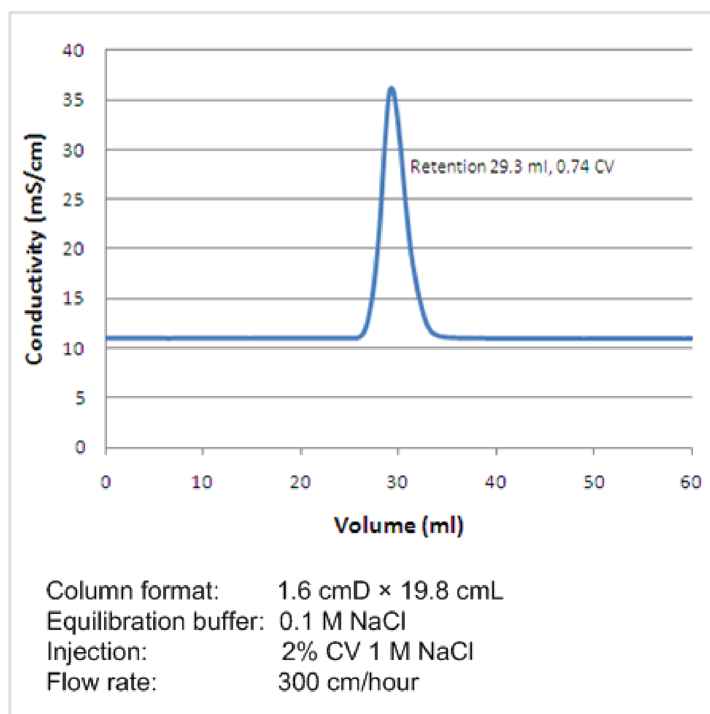

Figure 2 POROS™ column qualification

Example evaluation protocol for purification of mRNA using packed columns

This protocol can be used as a starting point to optimize the evaluation protocol for packed columns. Refer to the following sections for optimization guidance. Degas solutions before use to avoid gassing out during operation.

1. Pack and qualify the column as specified in this document.
2. Equilibrate the column using 3–4 CVs of 10 mM Tris-HCl, 0.5 M NaCl, 1 mM EDTA, pH 7.4.
3. Prepare the sample:
   a. Adjust the mRNA sample mix to approximately 10mM Tris-HCl, 0.5M NaCl, 1mM EDTA, pH 7.4.
   b. If denaturing is required, heat the sample at 65°C in a water bath for 10–15 minutes, then immediately place the sample on wet ice.
4. Load the sample onto the column at 50–150 cm/h.
5. Wash the column with an additional 2–3 CVs of 10 mM Tris-HCl, 0.5 M NaCl, 1 mM EDTA, pH 7.4.
6. Wash the column with 3–5 CVs of 10 mM Tris-HCl, 100–300 mM NaCl, 1 mM EDTA, pH 7.4, or until the conductivity stabilizes.
7. Elute the bound mRNA using 3–5 CVs of 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, or 3–5 CVs water.

   Note: Elution may be enhanced by heating the elution buffer, elution water, or the column to 65°C.
8. Regenerate the column with 3–5 CVs water.
9. Sanitize column with 3–5 CVs of 0.1 M NaOH, then wash with 3–5 CVs of equilibration buffer.
10. Store the column in storage solution at 2–8°C.

Chromatography condition optimization

General considerations

Although all binding occurs through base-pairing between the polyA tail of the messenger RNA and the oligo dT sequences bound to the surface of the resin, the optimal binding and elution conditions can vary due to a number of mRNA characteristics. Different mRNA load solutions operated with the same process conditions will yield variable results; therefore, standardized conditions or platform-type evaluations are not recommended. Test different loading and elution conditions to optimize capacity, separation, and yield based on the target molecule characteristics and process challenges.

Always filter the load through a 0.22 or 0.45 µm filter before loading to reduce fouling of the column screens. Degas solutions before use to avoid gassing out during operation.

Binding conditions considerations

- **pH**—Binding and destabilization of T-A pairs will occur at neutral pH
- **Buffer system**—Phosphate and tris buffers are commonly used. When choosing buffer systems, consider molecule stability, binding optimization, and the ability of the buffer to control pH in the desired operating range.
- **Conductivity**—Base pairing is promoted at elevated conductivity. At least 250–500 mM NaCl is typically used.
- **Flow rate**—The target operating flow rate is flexible, but optimal binding should be obtained with a residence time of ≥3 minutes (that is, ≤400 cm/H in a 20-cm length column).
- **Temperature**—Annealing to the surface bound polyT is performed at room temperature. If necessary, the total RNA load can be heated to 65–70°C to disrupt secondary structures.
- **Equilibration**—Column equilibration typically requires ~5 CV of loading buffer
- **Capacity optimization**—The maximum capacity depends on several factors, including mRNA size, sample solubility, load concentration and impurity composition, buffer composition, and conductivity.

Elution conditions considerations

Begin elution optimization with either a gradient elution from high to low salt (or water) or through a series of small steps, incrementally reducing the salt concentration of each step. After the elution concentration is determined, a series of larger steps can be implemented.

- **pH**—Use the same starting pH for load solution and equilibration buffer. If needed, optimize the pH of the elution buffer. For concentration determination of eluted mRNA, OD is pH-dependent.
- **Elution conductivity**—In the absence of salt, electrostatic repulsion occurs between the anionic backbones of the polyA and polyT causing the A-T pairs to separate. This separation allows elution of the polyA-mRNA. In some cases, elution can be achieved using water alone. Salt gradients are not typically performed. However, intermediate wash steps at reduced conductivity can improve impurity removal.
- **Recovery and yield** —mRNA yield is highly dependent on input titer, binding conditions, and fractionation. Recovery relies on the optimization of elution conditions for specific molecule characteristics.

Resin cleaning and storage

Clean the column

Clean the resin with 3 to 5 CVs of water or 20–30% alcohol followed by 3 to 5 CVs of 0.5 M NaOH as needed.

Different solutions may be required for column cleaning depending on the purity and composition of the sample feed.

Resin and packed column storage guidelines

- Store bulk resin at 2–8°C. Do not freeze.
- Store the resin in 18–20% ethanol at 2–8°C.
- The composition of the storage solution will impact the temperature at which freezing occurs. For example, 20% ethanol in water freezes at –9°C; 10% ethanol in water freezes at –4°C.

Note: Changing the storage temperature from room temperature to refrigerated temperature can affect packed bed stability due to buffer outgassing and storage solution volume changes. Allow the column to reach room temperature before use.

Troubleshooting

Observation	Possible cause	Recommended action
High backpressure	Presence of any amount of ethanol (shipping/storage solution) in the slurry or in the column	Fully exchange the ethanol before packing. Typically, this requires three exchanges.
	Compromised flow path: • Compressed sanitary gaskets • Closed, partially closed, or blocked inlet and outlet valves on the column • Improperly functioning valves on the chromatography system • Blocked inline filters	• Use narrow-bore sanitary gaskets. • Characterize the pressure of the entire chromatography system with no column in place, the system and empty column with the column outlet plumbed directly to waste, and the system and empty column with the column outlet plumbed back into the skid. • Ensure that the entire flow path is clear. • Change the inline filters.
	Clogged or very tiny frits (< 3 µm)	• Change or clean the frits (screens). • Run the column in upflow for 3 CVs, then downflow again. Observe if there is a change in pressure.
	Improperly scaled chromatography systems, including small-diameter tubing anywhere in the system and operating at the high end of the system range	• Verify that the skid pump and tubing diameters are scaled appropriately for the column operation and replace as needed. • Do not operate pumps at over ~70% of their capacity.
	Particle size gradient in the column caused by gravity settling the resin	Do not gravity-settle resin in the column before packing.
	Resin was frozen	Store the column at 2–8°C. Do not freeze.
Turbid column effluent after >3 CVs during packing	Column frits (screens) are too large for the resin (> 23 µm frit)	Use standard 10–23 µm screens (frits).
	Compromised flow adaptor o-ring, improperly assembled flow adaptor, or defective flow adaptor	Take the adapter apart, inspect all parts, and replace as needed.
Column qualification — high asymmetry	Column is underpacked; that is, the column is not packed at a high enough flow rate/ pressure	• Pack at a higher flow rate/pressure. • The top adapter position may need to be better seated in the packed resin bed to ensure that a headspace does not form.
	The system and plumbing allow for dilution of the salt plug	• Characterize a salt plug through the chromatography system at the qualification flow rate to understand how the plug moves through the system with no packed column in line. • Verify that the plumbing throughout the system (pre- and post-column) is consistent and that areas for dilution are minimized. • Verify that there is no air under the distributor.
	Salt injection method is not optimized	Verify that the desired amount of salt is loaded by checking the peak height and width. Ensure that the injection is consistent and applied as close to the column inlet as possible to minimize dilution from the system. The injection method should be well-described in your operating procedures to maintain reproducibility.
	The column needs more post-pack conditioning to stabilize the packed bed	Equilibrate the column with 2–3 CV of packing solution in downflow at the operating flow rate, 2–3 CV in upflow, and 2–3 CV in downflow again.
	2 M NaCl salt is used for the salt plug or an analyte interacts with the resin	Use recommended column qualification conditions.
Column qualification – low asymmetry	Column is overpacked or packed inconsistently	Repack the column following the recommended procedure.
	Water is used as the mobile phase	Add some salt to the mobile phase to reduce the charge interaction between the salt and the bead.

Observation	Possible cause	Recommended action
Column qualification – low asymmetry *(continued)*	Column not equilibrated long enough with sodium chloride before salt injection	Equilibrate ≥ 4 CVs if the packing solution is different from the qualification mobile phase.
Decreased performance: • Increased bandspreading • Decreased binding capacity • Decreased recovery • Increased pressure drop • Trace or “ghost” peaks during blank runs	Column fouling can occur due to precipitation of product or impurity, irreversible binding of lipid material, or other impurities	Clean the column.

Ordering information and product use

Table 3 Bulk resins

Cat. No.	Volume	Product use
A48605	10 mL	Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
A48606	25 mL	
A47382	50 mL	
A47383	250 mL	
A47384	1,000 mL	
A47385	5,000 mL	
A47386	10,000 mL	

Table 4 Prepacked formats

Product	Cat. No.	Volume	Product use
POROS™ Oligo dT(25) RoboColumn™	A48349	200 µL	For Research Use Only. Not for use in diagnostic procedures.
POROS™ Oligo dT(25) RoboColumn™	A48350	600 µL	
POROS™ Oligo dT(25) GoPure™ Column	A48607	0.5 cmD × 1 cmL (0.2mL)	
POROS™ Oligo dT(25) GoPure™ Column	A48352	0.5 cmD × 5 cmL (1mL)	
POROS™ Oligo dT(25) GoPure™ Column	A48351	0.8 cmD × 10 cmL (5 mL)	

Support

For service and technical support, go to **thermofisher.com/poros** or call toll-free in US: 1.800.831.6844.

For the latest service and support information at all locations, or to obtain Certificates of Analysis or Safety Data Sheets (SDSs; also known as MSDSs), go to **thermofisher.com/support**, or contact you local Thermo Fisher Scientific representative.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. 100092591

Revision	Date	Description
B	18 May 2020	Add A48605, A48606, A48349, A48350, A48351, A48352, A48607. Change bulk resin product use statement to Pharmaceutical Grade Reagent. Update manufacturer address.
A	01 April 2020	New document for POROS™ Oligo (dT)25 Affinity Resin.

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