Scalable mRNA Affinity Capture

Supporting Development and Production of mRNA-Based Therapeutics

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Messenger RNA (mRNA) has significant therapeutic potential, as evidenced by rapidly expanding pipelines with clinical assets targeting many different indications. Given the breadth of potential applications, an efficient and scalable purification platform will be essential to supporting the growth and success of the mRNA field. The workflow for producing an mRNA-based therapeutic comprises DNA template preparation, mRNA synthesis based on that template, and downstream mRNA purification. Chromatography is a critical unit operation for both plasmid and mRNA purification, and multiple approaches can be considered, each with advantages and pitfalls.

A Resin for mRNA Capture

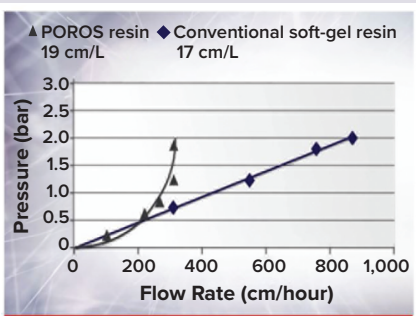
Thermo Fisher Scientific developed POROS Oligo dT(25) affinity resin to isolate and purify mRNA and to support commercial production of mRNA-based therapeutics. The resin consists of a 50-µm porous poly(styrene-*co*-divinylbenzene) base bead with a polydeoxythymidine (poly-T) 25-mer (dT-25) conjugated to the surface using a proprietary linker. Figure 1 highlights advantages of the POROS bead's backbone, size, and large throughpores.

After in vitro transcription (IVT), the resin is applied to capture polyadenylic acid (poly-A)–containing mRNA using a simple adenosine–thymine base-pairing mechanism. Because of the beads' specificity for poly-A tails, only full-length mRNA molecules remain following the capture step; process-related impurities such as DNA templates, nucleotides (nt), enzymes, and buffer components are removed. An additional polishing step can be performed to remove double-stranded RNA (dsRNA), uncapped RNA, and secondary RNA structures (e.g., short hairpin RNA, shRNA) from the final drug substance.

POROS Oligo dT(25) affinity resin has a dynamic binding capacity of up to 5 mg/mL for a 4,000-nt mRNA sequence and can achieve product recoveries of >90%. It enables purification under native conditions, exhibits high selectivity, provides scalability, and lacks animal-derived components. The resin can serve as a purification platform for all mRNA molecules that contain a poly-A tail. Column sizes can range from a few milliliters to hundreds of liters. Purification conditions can be optimized during experiments in 96-well plates or in miniaturized columns for automated chromatography, then verified at bench scale with prepacked columns, and ultimately applied at larger scales.

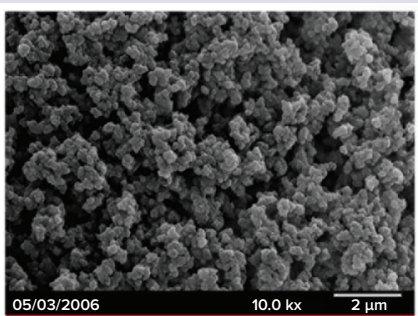
Figure 2 presents a chromatogram from purification of 2,000-nt mRNA using POROS Oligo (dT)25 affinity resin. The sample loaded onto the column was a post-IVT mixture containing single- and double-stranded mRNA as well as residual DNA and enzymes from the production process. The orange line represents the UV 260 nm absorbance measurement and shows the sample's chromatographic profile. Conductivity across the run — including steps for sample loading, washing, elution, and column cleaning — is shown in blue.

Figure 1: Key features of the POROS chromatography resin



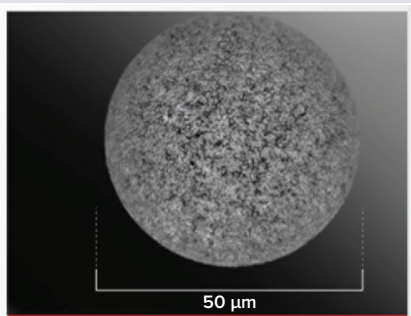
Poly(styrene-*co*-divinylbenzene) Backbone

- Linear pressure-flow curve
- Rigid, linear, scalable performance
- Easy handling
- High robustness and chemical stability



Large Throughpores

- Reduced mass-transfer resistance
- Good maintenance of capacity and resolution across linear velocities
- Efficient purification



50-µm Bead Size

- Superior resolution
- Improved capacity through novel surface chemistries
- Excellent pressure-flow properties
- Full scalability

Figure 2: Chromatogram showing efficient separation of a 2000-nt mRNA from an in vitro transcription (IVT) mixture at a load concentration of 2 mg/mL; elution using water yielded >95% recovery.

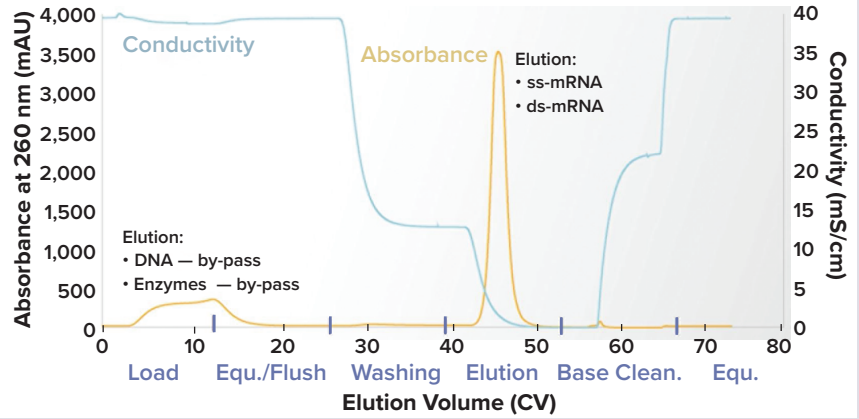
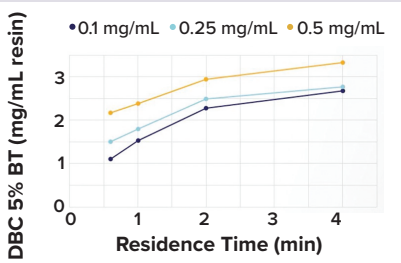


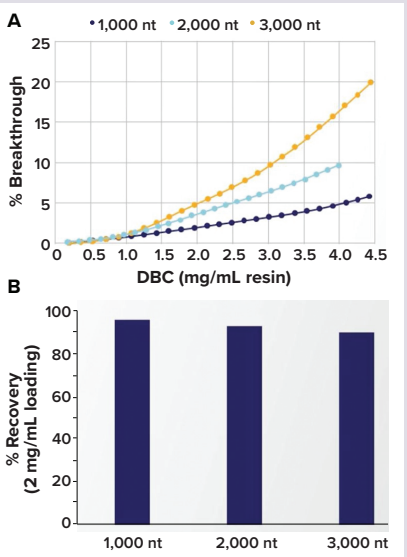
Figure 3: Dynamic binding capacity (DBC) determination for a 3000-nt mRNA feed at different concentrations and load residence times; BT = breakthrough



Residence Time for mRNA Load (flow rate):

0.6 min (300 cm/hour)	2.0 min (90 cm/hour)
1.0 min (180 cm/hour)	4.0 min (45 cm/hour)

Figure 4: mRNA molecule size influences resin binding capacity (A) but not final recovery (B).



Absorbance values increased early in the run, indicating that DNA and other components were flushing through the column. The step elution down to 150 mM NaCl helped to elute small, truncated poly-A components that bound weakly to the column and to the mRNA itself. The subsequent transition into water provided a sharp, narrow mRNA elution peak. A small peak was observed in the NaOH-base cleaning step, representing removal of residual components from the column.

POROS Oligo (dT)25 resin has shown consistent dynamic binding capacity (DBC) over 70 reuse cycles (data not shown). It also remains stable in a breadth of pH (1–13), cleaning, and storage conditions.

Table 1: Upper limit of salt concentration, by mRNA length

mRNA Size	NaCl (M)	KCl (M)
3,000 nt	1.4	2.0
2,000 nt	1.8	2.0
1,000 nt	1.4	1.8

Considerations for Optimizing Capture

Precipitation Point Determination: Precipitation of mRNA differs by construct size and sequence, as well as by the type and concentration of salt used during the capture step. High salt concentrations can induce formation of dsRNA. A 2,000-nt mRNA molecule begins to precipitate at 1.4 M NaCl. Loading a column above that level can diminish recovery (Table 1).

DBC Determination: The DBC of a capture step must be calculated to determine how much product to load onto a column. In terms of mass, mRNA concentration is relatively low compared with that of monoclonal antibodies (MAbs) and other proteins. Load concentration can influence a resin's DBC because the binding is diffusion limited. Diffusion is driven by a concentration gradient or difference between the load material and the resin. If the concentration in the load material is low, there is less of a driving force to get the mRNA onto the resin.

To determine the POROS Oligo (dT)25 product's DBC, a breakthrough curve was generated for three mRNA load concentrations at four residence times (Figure 3). For all concentrations, DBC at 5% breakthrough showed little increase between two and four minutes of residence time. Thus, purification can be run at a two-minute residence time without sacrificing resin capacity.

Impact of Molecule Size: The size of an mRNA construct influences resin DBC but not final recovery. Figure 4 shows that >90% recovery can be achieved for mRNA molecules of 1,000, 2,000, and 3,000 nt despite differences in resin binding capacity.

Elution Conditions: Because mRNA binds to the POROS Oligo (dT)25 resin in high ionic-strength conditions at neutral pH, most constructs can be eluted from a column using solutions of low ionic strength, including water. However, alternative elution buffers such as citrate and tris can be considered when use of water does not result in sufficient recovery or mRNA stability. A comparison of different citrate and tris elution buffers has demonstrated that 1 mM citrate at pH 6.0 is a suitable alternative to water, if necessary (data not shown).

Furthering the Field

Regulatory approval of mRNA-based COVID-19 vaccines in 2021 amplified interest in using mRNA for cancer vaccination, allergy tolerization, protein replacement, and gene editing. Success of the field will depend on availability of innovative technologies such as POROS Oligo (dT)25 affinity resin to establish a robust, scalable production platform.

Now with Flagship Pioneering, ***Jenny England,*** *PhD, was R&D manager in the innovation and applications group at Thermo Fisher Scientific. Please send inquiries to Dave Humphries; david.humphries@thermofisher.com.*

POROS resins are pharmaceutical-grade reagents designed for manufacturing and laboratory use only.