Accelerating mRNA-based therapy development with scalable purification of *in vitro* transcribed mRNA

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Bioprocessing

INTRODUCTION

The diversity of potential mRNA-based therapies has led to increased interest in using synthetic mRNA as a tool in the treatment of multiple diseases, such as cancer, stem cell therapies and infectious diseases. Nevertheless, obtaining larger quantities of synthetic mRNA for clinical treatment remains a challenge. Current mRNA production methods require multiple purification steps and various chemicals, thereby creating a bottleneck for large-scale manufacturing of *in vitro* transcribed (IVT) mRNA. To overcome these challenges, a new affinity chromatography resin for the isolation and purification of mRNA was developed.

THERMO SCIENTIFIC™ POROS™ OLIGO (dT)25 AFFINITY RESIN

- ✓ Simple mRNA capture through AT base pairing
- ✓ Easy to use: load in NaCl and elute in water
- ✓ Dynamic Binding Capacity up to 5 mg/mL for 4000nt mRNA
- ✓ > 90% recovery
- ✓ Excellent scalability
- ✓ Non-animal derived

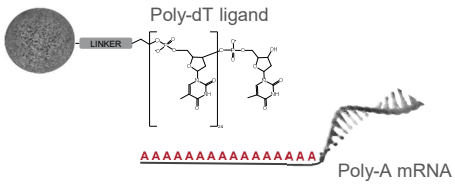
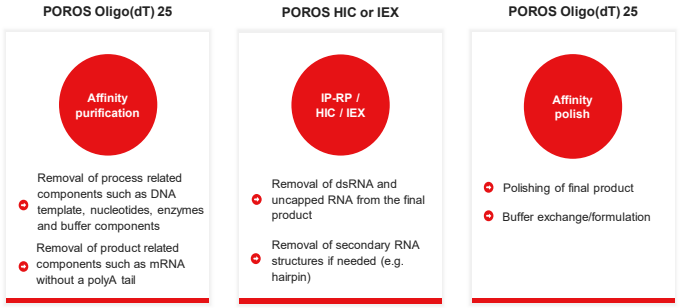


Fig. 1 POROS Oligo (dT)25 affinity resin consists of a poly-deoxythymidine (dT-25) ligand attached to a 50µm rigid, porous bead through a proprietary linker. The poly-dT ligand allows binding with poly-A tailed mRNA molecules through AT base pairing.

POROS Oligo (dT)25 for mRNA production



- ✓ Designed for the isolation and purification of mRNA from *in vitro* transcription manufacturing processes
- ✓ Simplified workflow to maximize efficiency and reduce complexity of subsequent polish steps

OPTIMIZING PROCESS CONDITIONS FOR RESIN USE

Precipitation point determination

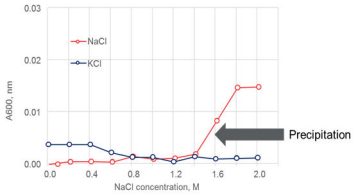


Fig. 2 mRNA salt tolerance to precipitation. Precipitation of 2000nt mRNA at >1.4M NaCl. Salt tolerance is dependent on mRNA size and sequence, type of salt and concentration.

Salt type and concentration

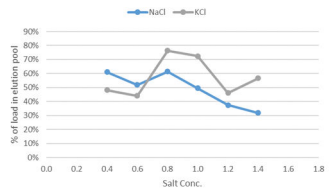


Fig. 3 Effect of salt type and concentration on mRNA binding during screening. This graph shows recovery data after static binding of a 2000nt mRNA molecule.

- ✓ Determine precipitation point to optimize recovery
- ✓ Recovery and yield (% load in elution pool) are dependent on the type of salt and concentration used.
- ✓ Compatible with a range of buffer and elution salts

RESIN PERFORMANCE – ELUTION, RECOVERY & PURITY

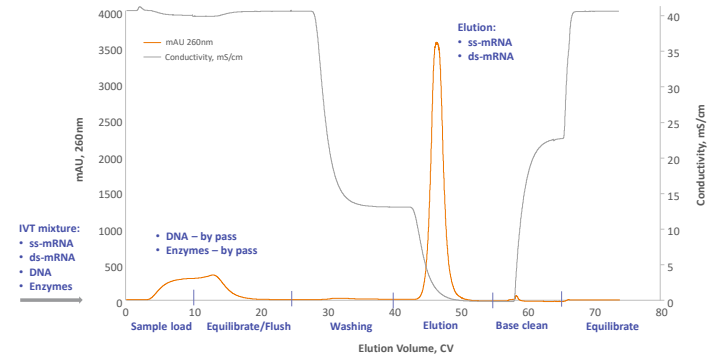


Fig. 4 Chromatogram showing efficient separation of a 2000nt mRNA from an IVT mixture at a load concentration of 2 mg/mL. Elution was performed using H_2O and yielded in >95% recovery.

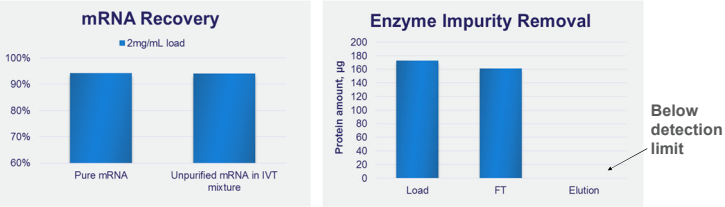


Fig. 5 High recovery and purity independent of sample type used. Recovery of mRNA from pure mRNA and unpurified IVT mixture, showed no differences (left). Amount of protein was determined in load, flow through (FT) and elution pools (right). No proteins were detected in the elution pool, indicating excellent impurity removal of IVT mixture products.

The POROS Oligo (dT)25 affinity resin demonstrates:

- ✓ Efficient elution at different load concentrations
- ✓ Excellent recovery with high purity independent of sample type used

Impurity analysis by HPLC

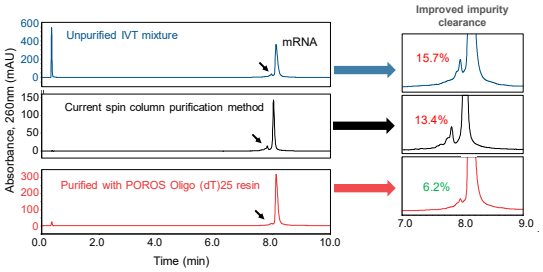


Fig. 6 Efficient removal of impurities compared to the spin column method. HPLC analysis of unpurified IVT mixture, spin column purified mRNA and mRNA purified with POROS Oligo (dT)25 resin.

- ✓ Purification with POROS Oligo (dT)25 resin leads to significant reduction of impurities

CONCLUSIONS

The POROS Oligo (dT)25 resin addresses the current challenges involved with large scale mRNA purification used in potential mRNA-based therapies.

Use of this resin will:

- ✓ Simplify your mRNA downstream process
- ✓ Increase purity and yield
- ✓ Allow for scalable mRNA purification process without the use of toxic chemicals

Customer testimonial – *"This promising technology will allow us to meet the increasing demands of mRNAs from our customers"* – Peter Scheinert, CEO AmpTec

Samples used were kindly provided by AmpTec