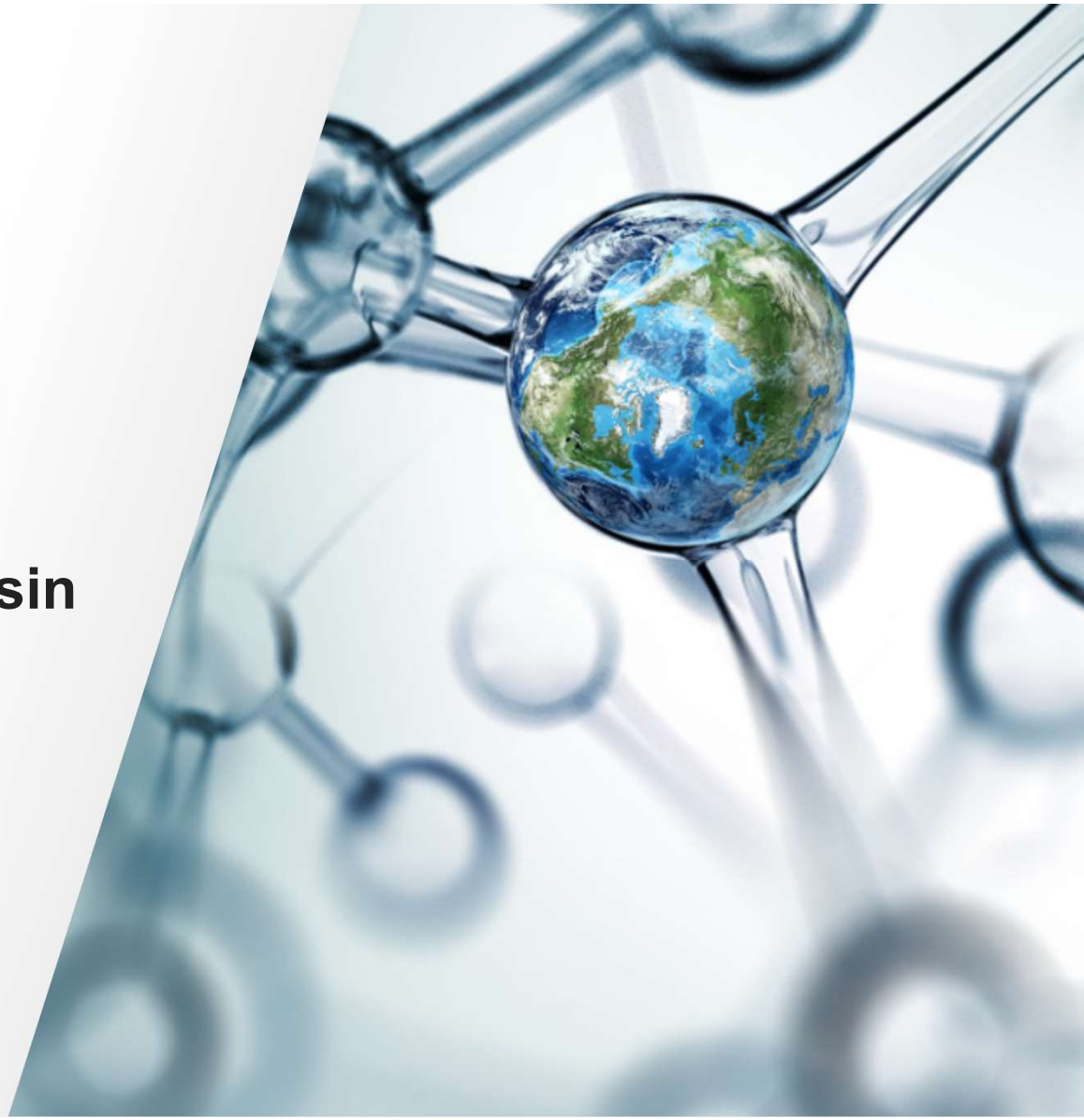
ThermoFisher SCIENTIFIC

Scalable purification of in vitro transcribed mRNA with POROS Oligo (dT)25 affinity resin



The world leader in serving science

Leading Capabilities for Every Step of Your Workflow

QC and Analytics

Cell culture development | Scale up to production | Harvest and collection | **Purification** | Bulk storage and final fill

Production Materials and Logistics Services

- CaptureSelect™ resins: The largest portfolio of affinity resins, for purification of every antibody format, biosimilars/biobetters and viral vectors
- POROS™ resins: Portfolio of IEX, affinity and HIC resins with unique features, improving the resolution, capacity and yield of processes
- Products used in many commercial and late stage processes
- State of the art manufacturing facilities:
  - Affinity ligand production: upstream vessels ranging from 10L- 15,000 L
  - Large scale resin manufacturing: commercial lot sizes of 250L
- Custom resin development programs offering tailored purification solutions

POROS™ chromatography resins

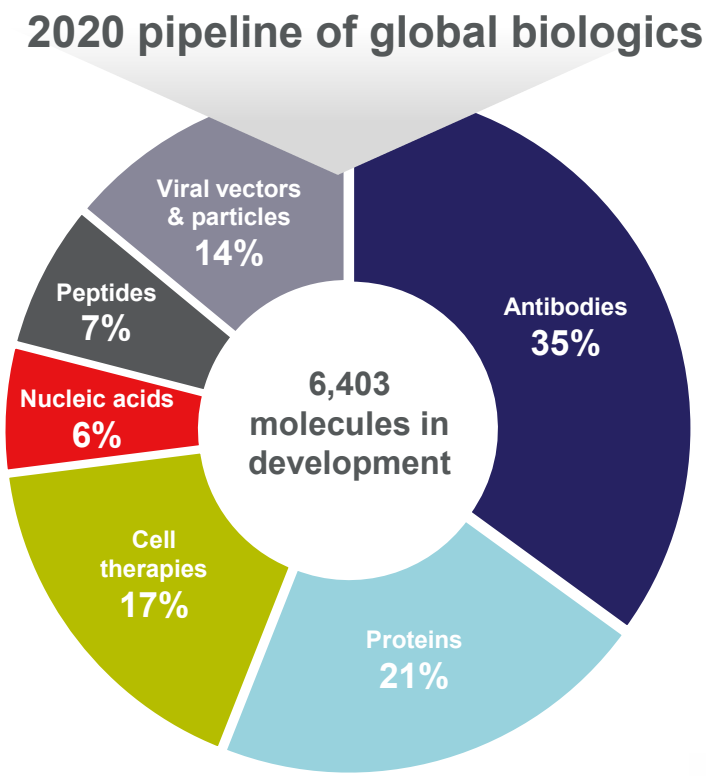
CaptureSelect™ affinity ligands and resins

Transfer assemblies

Large volume liquids

Host Cell DNA, Host Cell Protein and Protein A Quantitation

Growing Diversity of Biological Molecules in Development

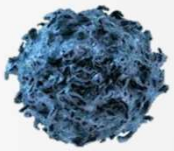



Nucleic acids


Virus


Therapeutic protein


Fab fragment

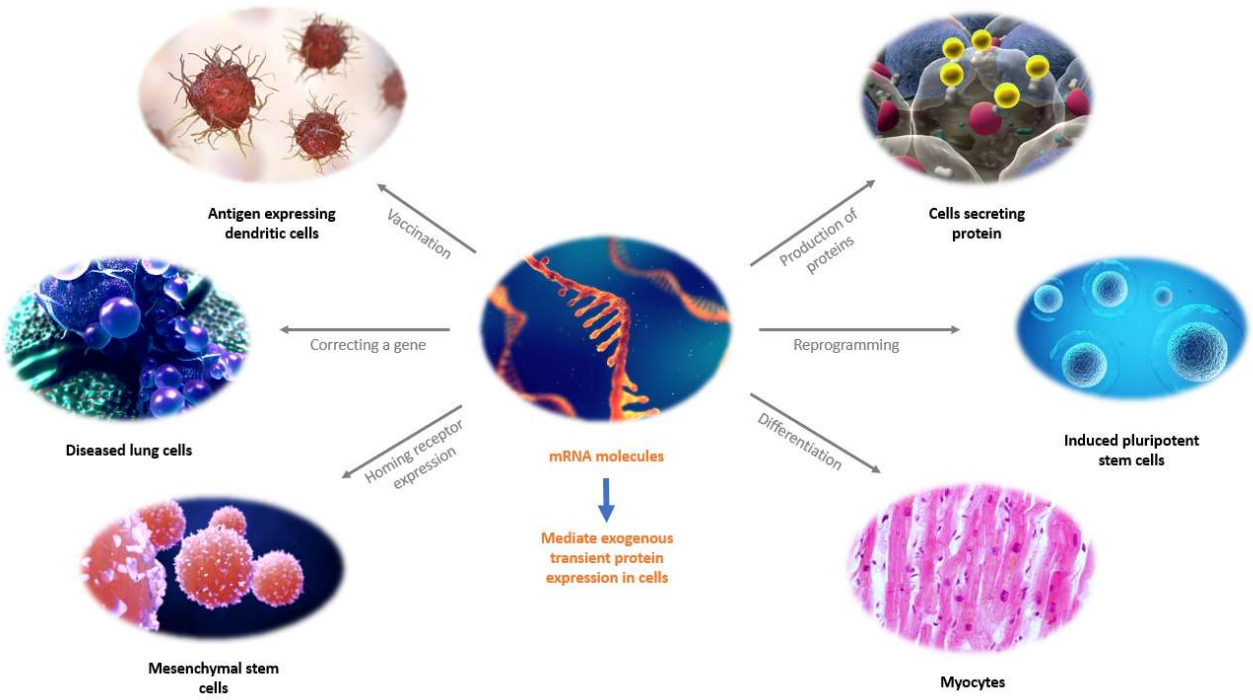

T cells


Antibody therapeutics

Source: Pharmaprojects, January 2020

New molecule modalities lead to new purification challenges driving a need for additional tools

Synthetic mRNA applications are diverse



Vaccines and therapies for:

- Oncology
- Rare and common infectious diseases
- Protein replacement therapies

Obtaining larger quantities of synthetic mRNA for clinical treatment remains a challenge

Thermo Scientific™ POROS™ Oligo (dT)25 Affinity Resin

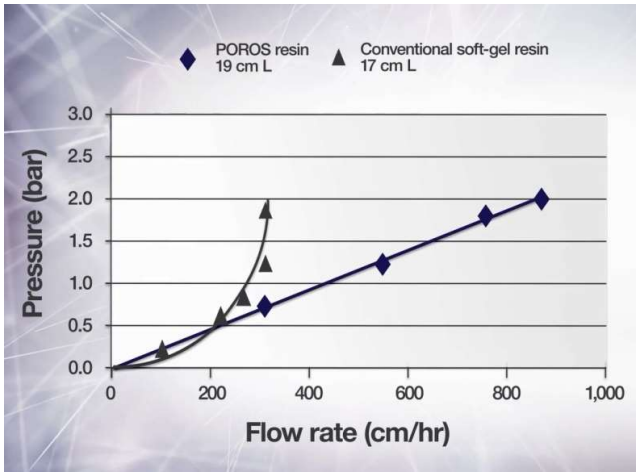
Resin features and benefits

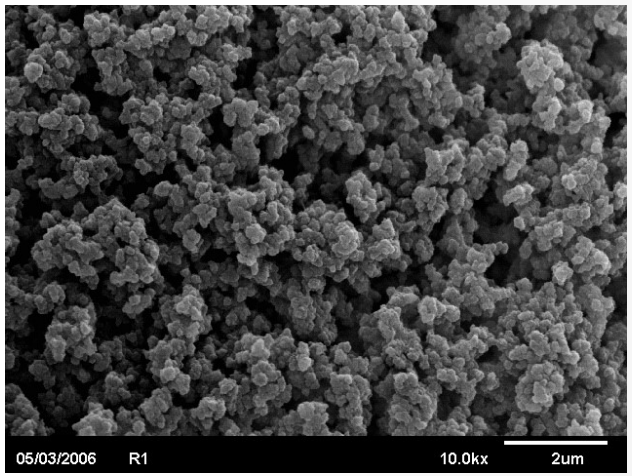
- Removes process impurities such as enzymes and DNA
- Simplified workflow helps to maximize efficiency, thereby reducing complexity of subsequent polish steps
- Easy to use: Load in NaCl, Elute in water
- Excellent scalability
- Non-animal derived

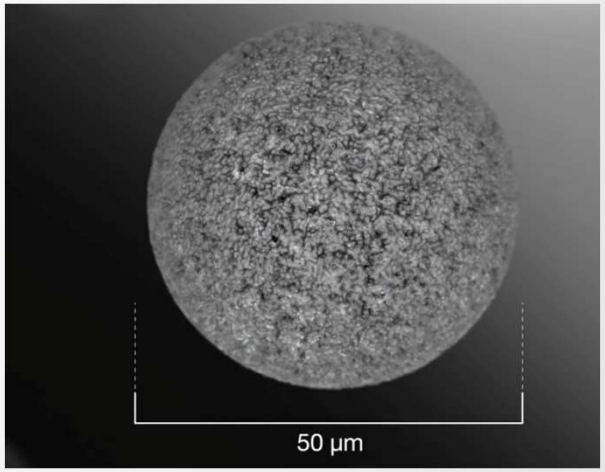
- Polythymidine (poly-dT) 25-mer with proprietary linker
- Simple mRNA capture through AT base pairing
- Large-pore structure of POROS beads deliver superior flow characteristics
- DBC up to 5 mg/mL for 4000 bp mRNA
- >90 % recovery
- Physically robust and reusable:
  - high salt • high pH • high temperature
- Available in bulk formats: 10, 25, 50, 250 mL, 1, 5, 10 L
- Prepack formats available

Designed for the purification and isolation of mRNA from in vitro transcription manufacturing processes

The Unique Features of the POROS™ Bead







Poly(styrene-divinylbenzene) Backbone

- Linear pressure flow curve
- Rigid, linear and scalable performance
- Easy handling
- Highly robust and chemically stable

Large throughpores

- Reduced mass transfer resistance
- Capacity and resolution well maintained over a wide range of linear velocities
- More efficient purification

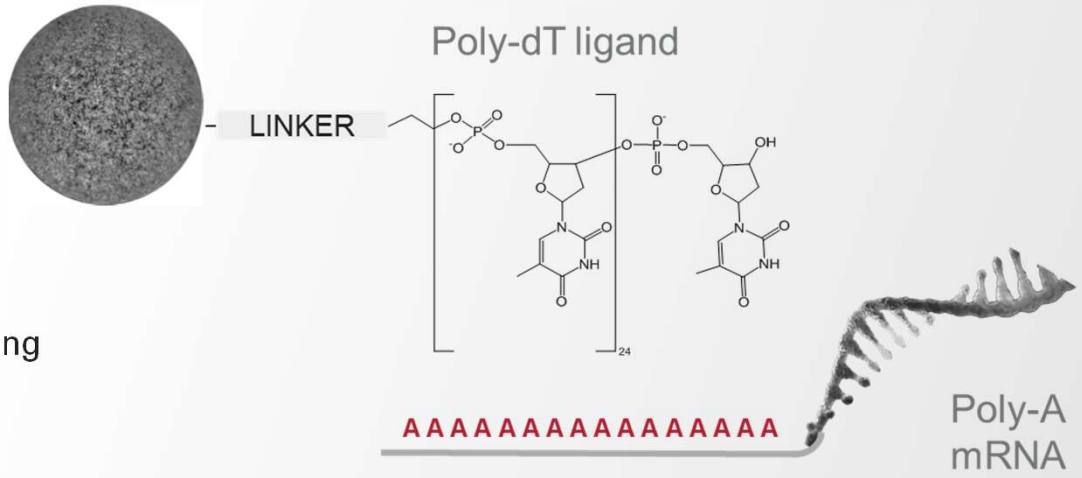
50 micron bead size

- Superior resolution
- Improved capacity through novel surface chemistries
- Excellent pressure-flow properties
- Fully scalable

Thermo Scientific™ POROS™ Oligo (dT)25 Affinity Resin

Resin technical features

- Based on POROS technology
  - Designed for the purification of biomolecules
- 50µm rigid, porous bead
  - Pore size ~200nm
  - Poly(styrene-co-divinylbenzene) base bead
  - Coated with proprietary functional hydrophilic coating
- Ligand with proprietary linker
  - dT-25 – poly-deoxythymidine



POROS Oligo (dT)25 for mRNA production

POROS Oligo(dT) 25

Affinity purification

- Removal of process related components such as DNA template, nucleotides, enzymes and buffer components
- Removal of product related components such as mRNA without a polyA tail

POROS HIC or IEX

IP-RP / HIC / IEX

- Removal of dsRNA and uncapped RNA from the final product
- Removal of secondary RNA structures if needed (e.g. hairpin)

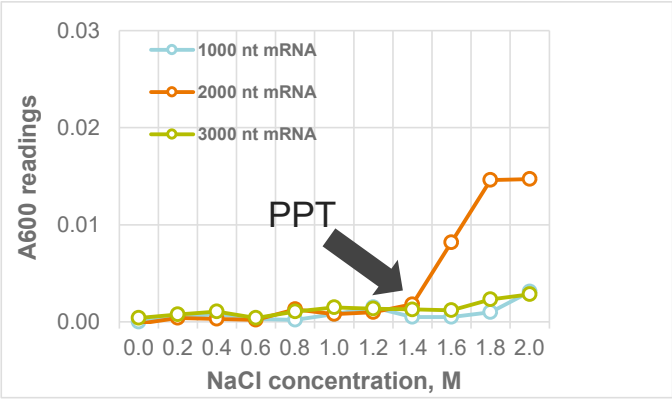
POROS Oligo(dT) 25

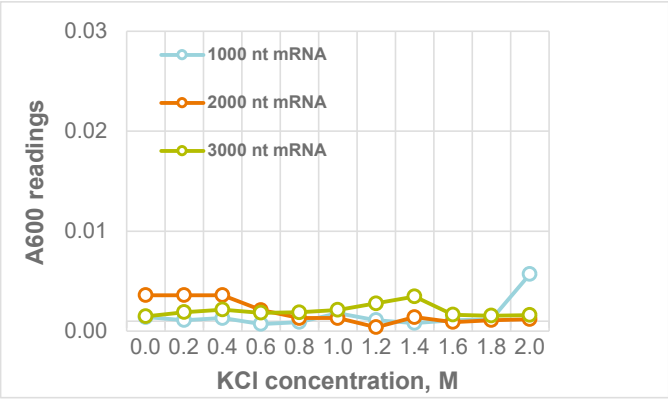
Affinity polish

- Polishing of final product
- Buffer exchange/formulation

mRNA precipitation point determination

Precipitation of mRNA at increasing salt concentrations





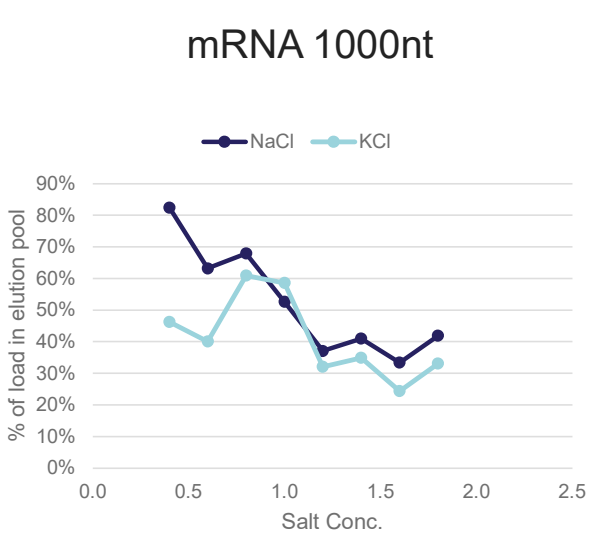
A600 measurement – optical density

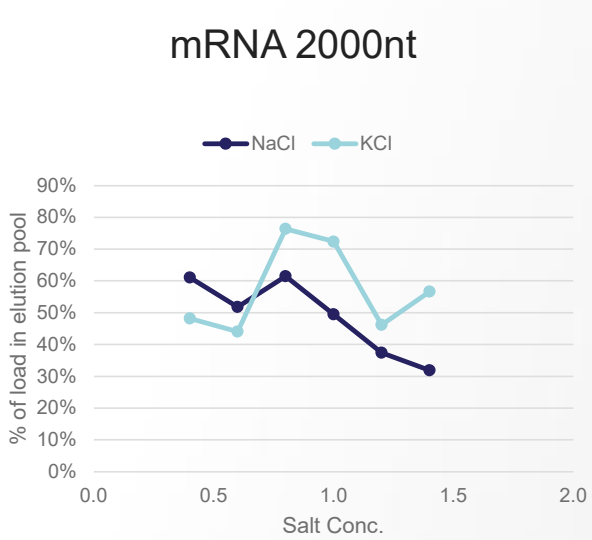
Salt concentration

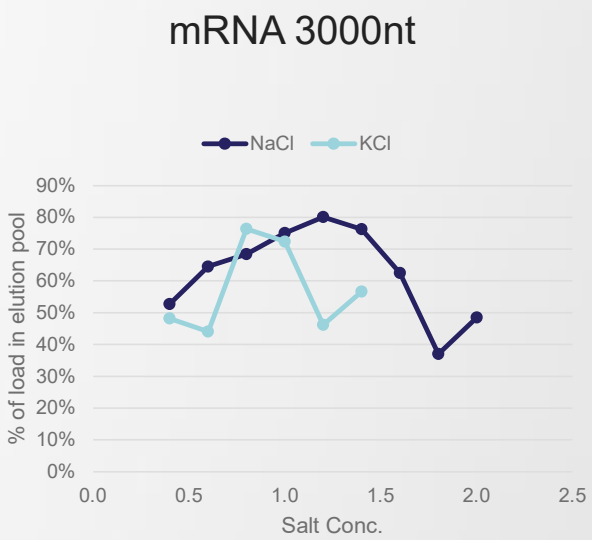
mRNAs	NaCl (M)	KCl (M)
3000 nt	1.8	2.0
2000 nt	1.4	2
1000 nt	1.8	1.8

mRNA precipitation is dependent on construct size and sequence, type of salt and concentration

Salt Type & Concentration effect on mRNA Binding during screening



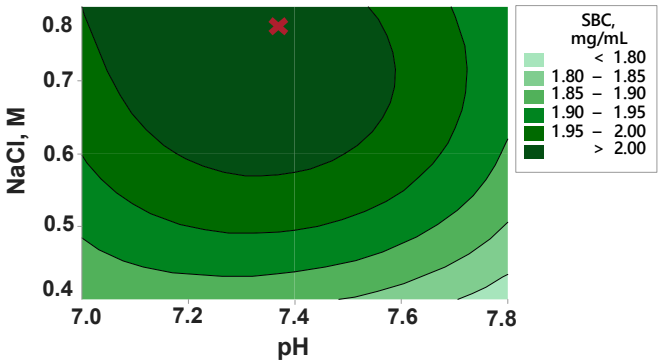




POROS Oligo (dT)25 resin shows efficient elution over a wide range of salt load concentrations

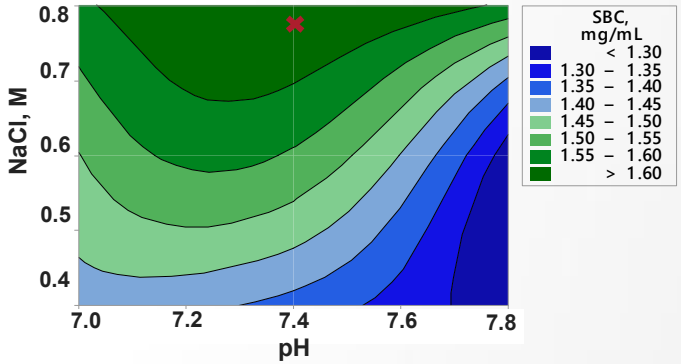
Optimization of salt and pH – Contour plots of static binding capacity

mRNA 1000 nt in Tris buffer



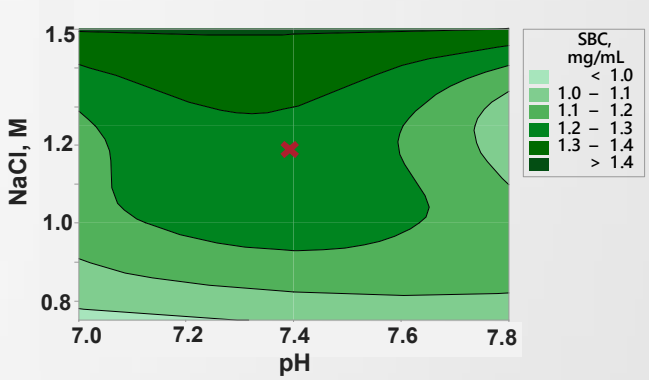
✖ 10mM Tris, 1mM EDTA, 0.8M NaCl, pH 7.4

mRNA 2000 nt in Tris buffer



✖ 10mM Tris, 1mM EDTA, 0.8M NaCl, pH 7.4

mRNA 3000 nt in Tris buffer

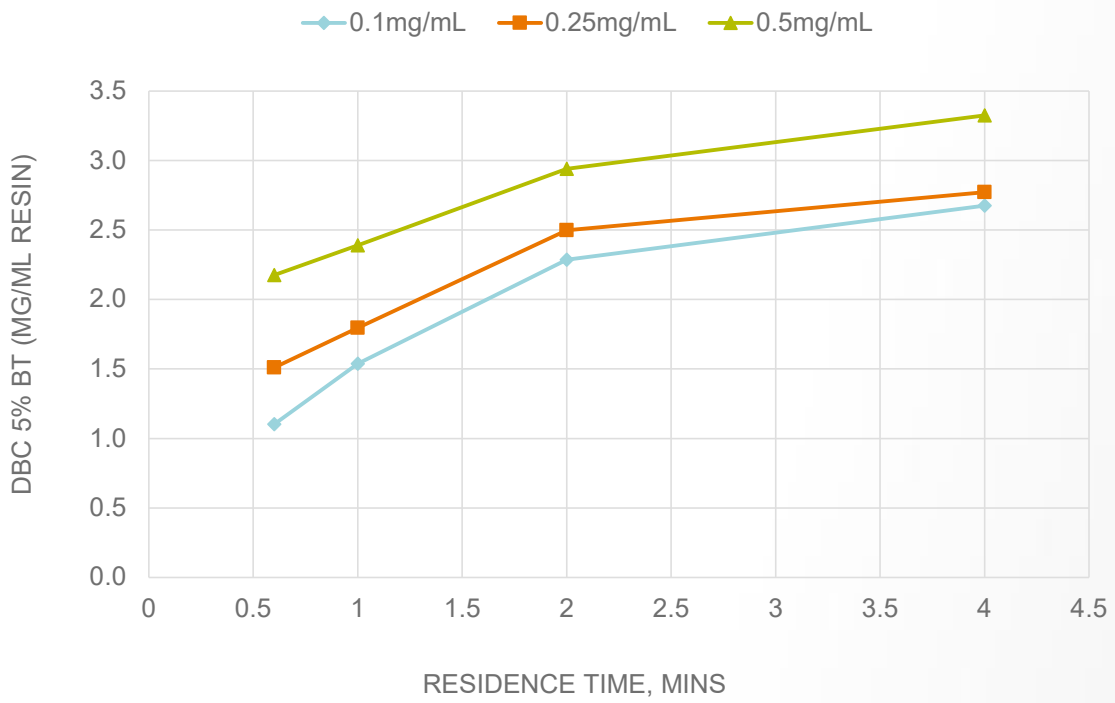


✖ 10mM Tris, 1mM EDTA, 1.2M NaCl, pH 7.4

Optimal binding conditions are construct dependent

Dynamic Binding Capacity study -

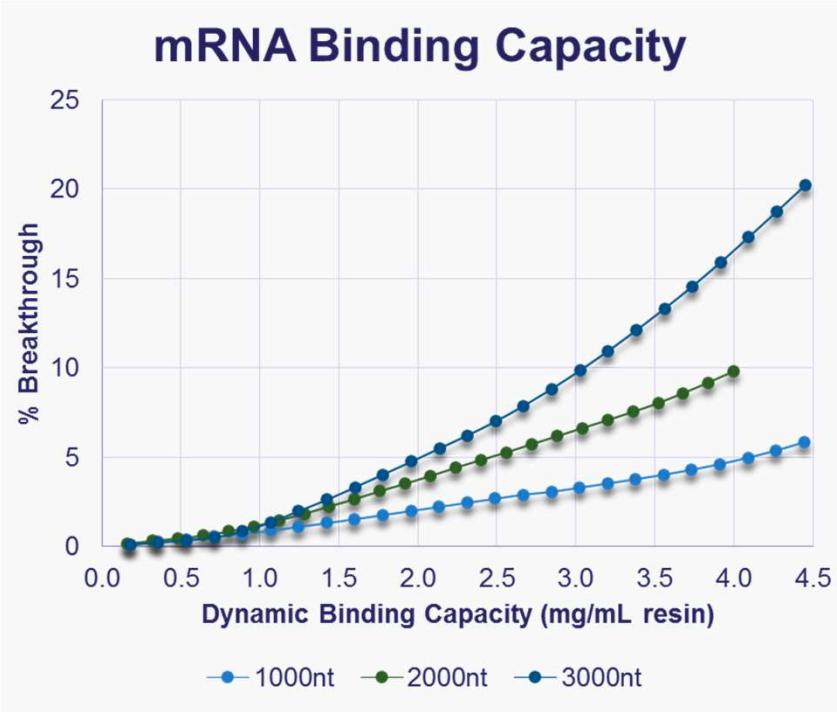
3000 nt mRNA feed concentration & load residence time

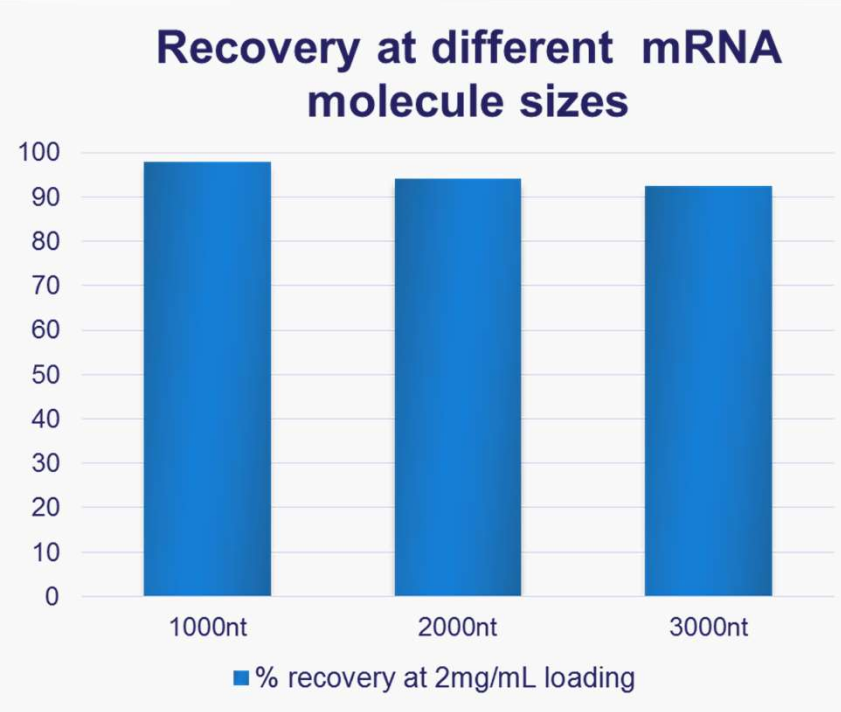


Residence time for mRNA load (Flow rate)
0.6 min (300cm/hr)
1.0 min (180cm/hr)
2.0 mins (90cm/hr)
4.0 mins (45cm/hr)

Binding capacity is increased through higher mRNA concentration in the load and residence time

Influence of molecule size – binding capacity and recovery

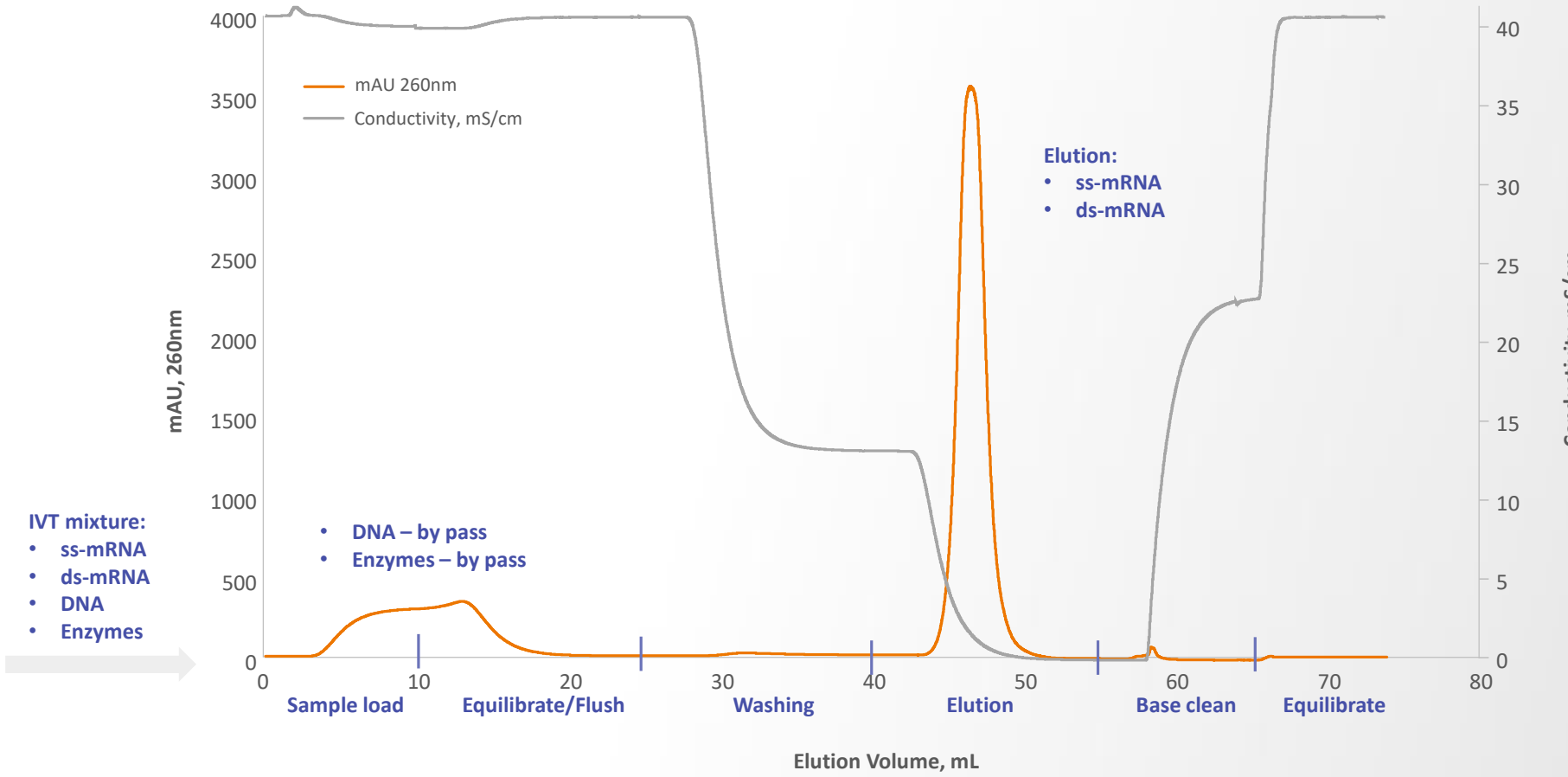




mRNA molecule size impacts binding capacity but not final recovery

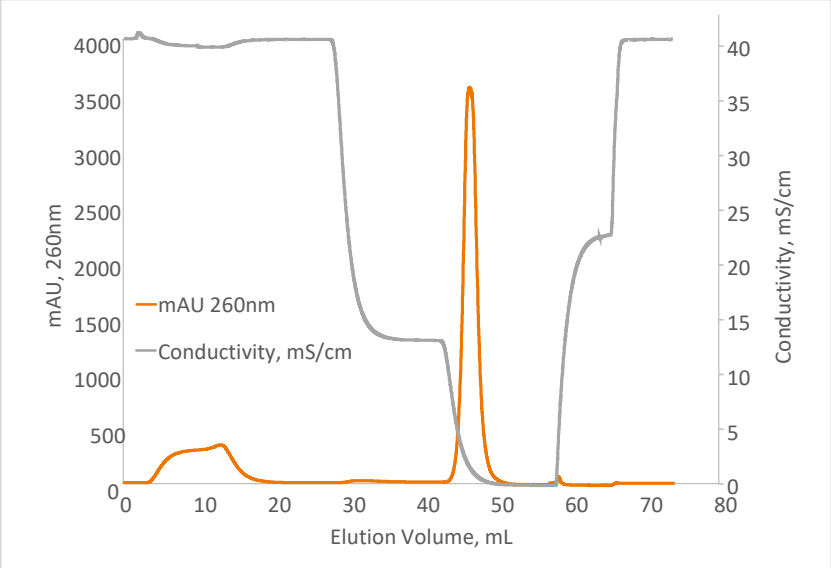
Purification of 2000nt mRNA from IVT mix – 2mg/mL Load

Samples provided by AmpTec Amplification Technologies

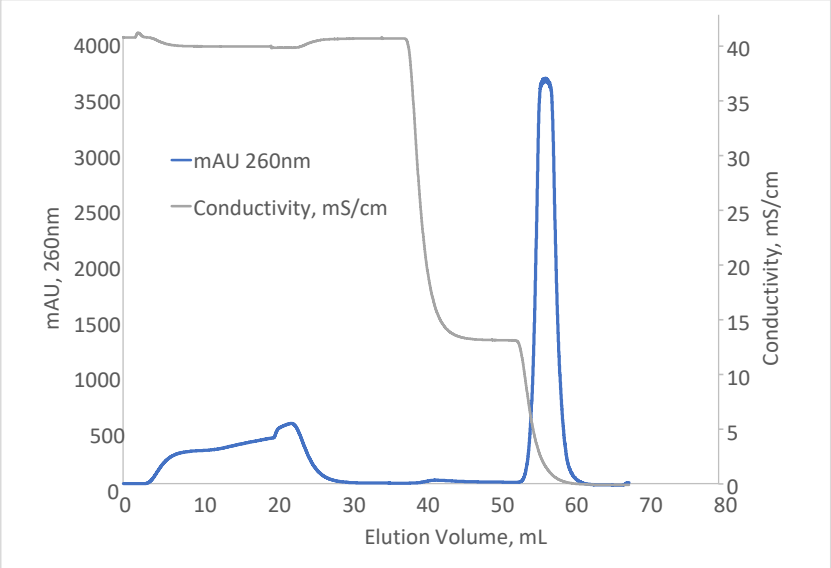


2000 nt mRNA Separation from IVT Mixture

mRNA IVT mixture load at 2mg/mL

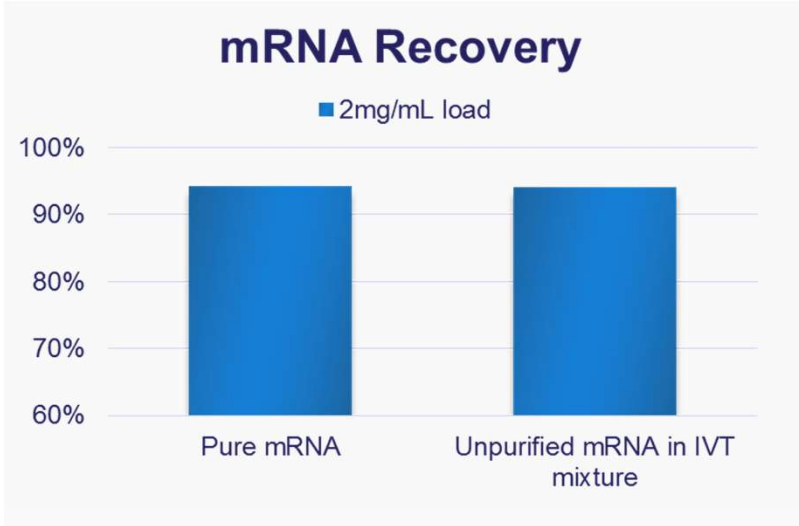


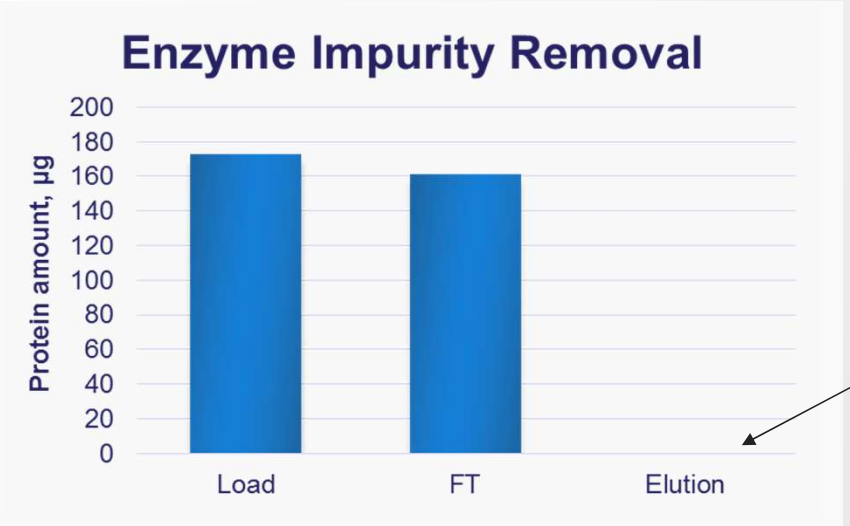
mRNA IVT mixture load at 4mg/mL



Excellent elution efficiency at different loading concentrations

Recovery and impurity removal

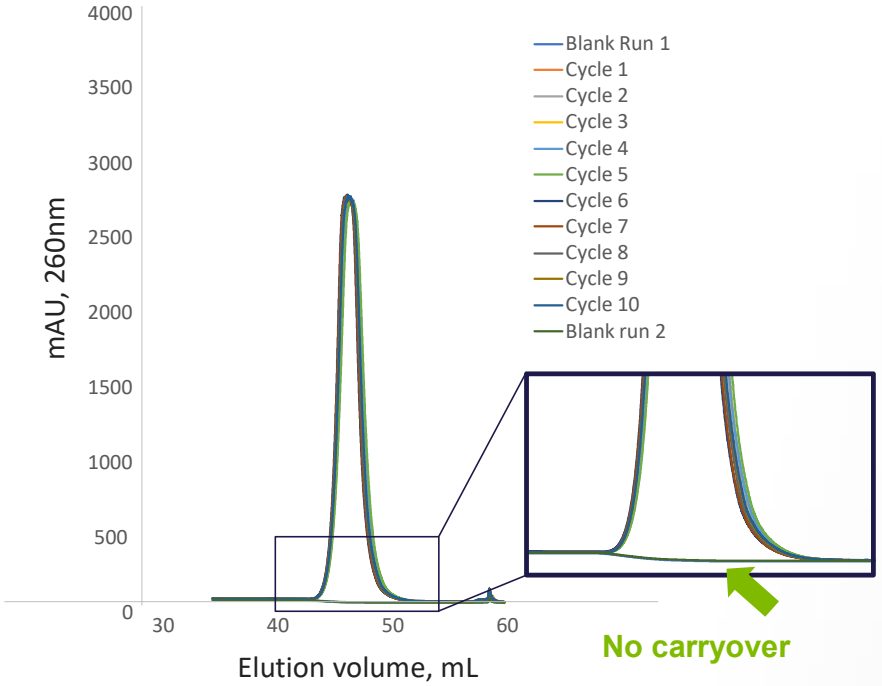


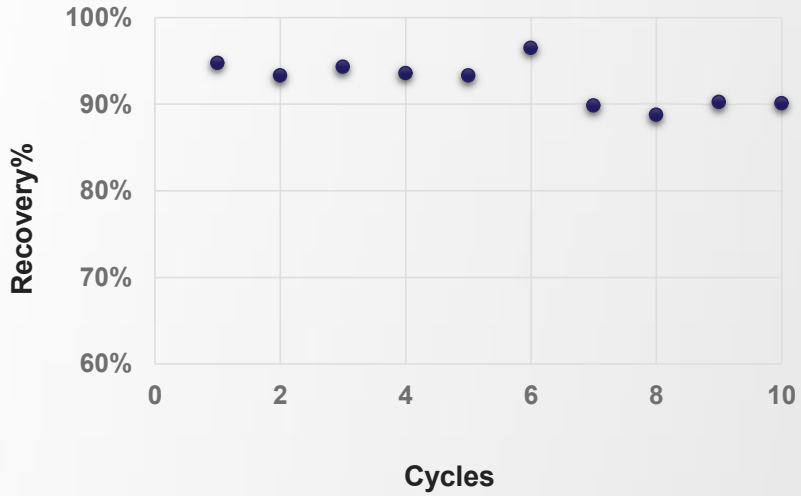


High recovery and purity independent of sample type

POROS Oligo (dT)25 affinity resin reuse

Purification of mRNA (1809nt + polyA 120nt) over multiple cycles from IVT mixture





Recovery is not impacted by resin reuse and cleaning

Partnering with AmpTec to Deliver Increasing Demand

Opportunity

- AmpTec, a leading RNA CMO, has increasing demand for large quantities of clinical-grade mRNA
- Needs efficient and scalable solution for large scale manufacturing projects

Our plan

- Worked with customer to understand challenges with current technologies: reverse phase HPLC won't scale and spin columns are inefficient
- Thermo Fisher offered POROS Oligo (dT)25 affinity resin and is supporting evaluation and platform process development

Results

- Adopting POROS Oligo (dT) into mRNA purification platform, allows customer to take on projects such as large scale COVID-19 vaccine manufacture
- "*This promising technology will allow us to meet the increasing demands of mRNAs from our customers*." - Peter Scheinert, CEO AmpTec

Purification of 2000nt mRNA from IVT mix – 2mg/mL Load

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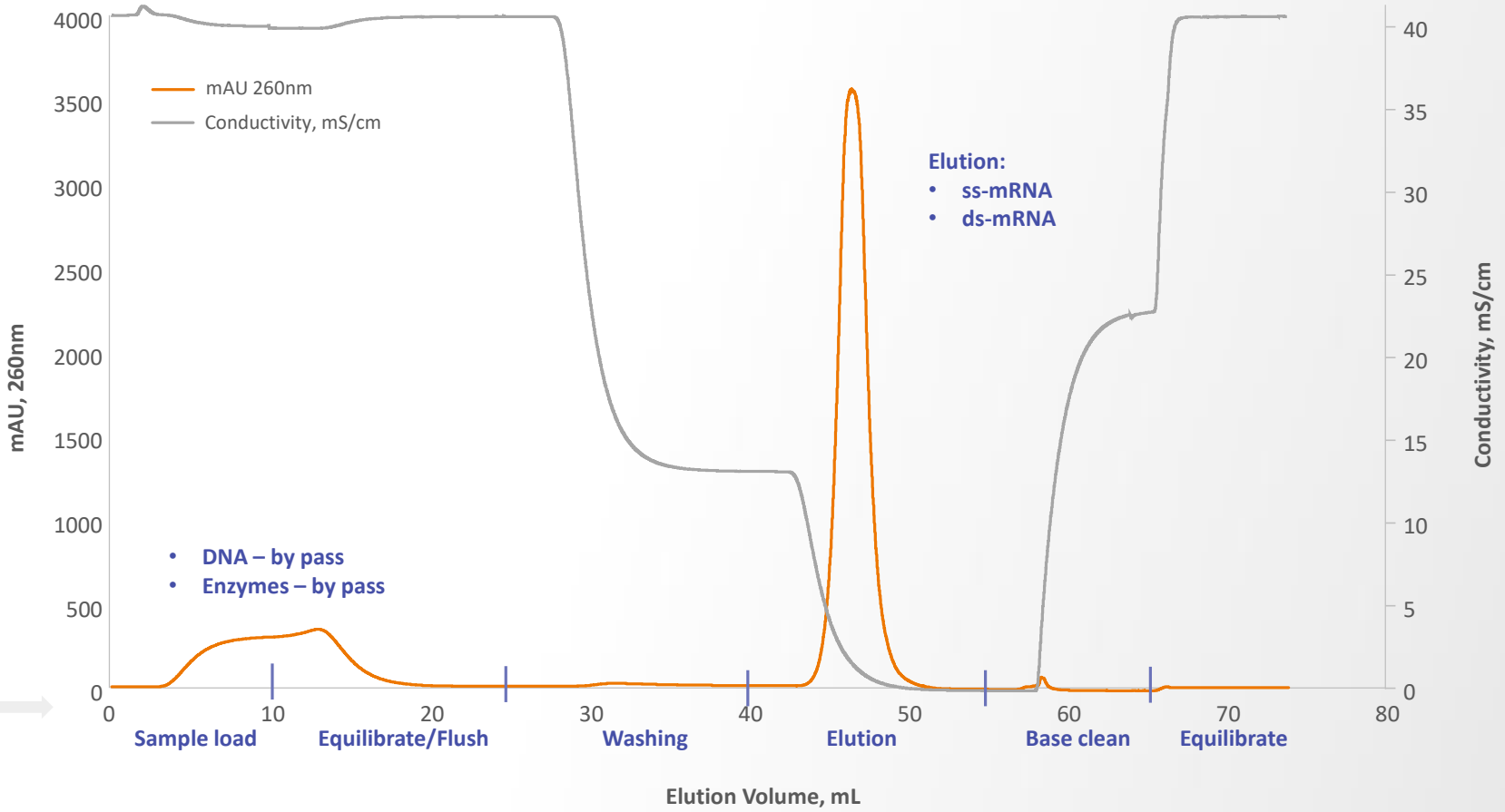
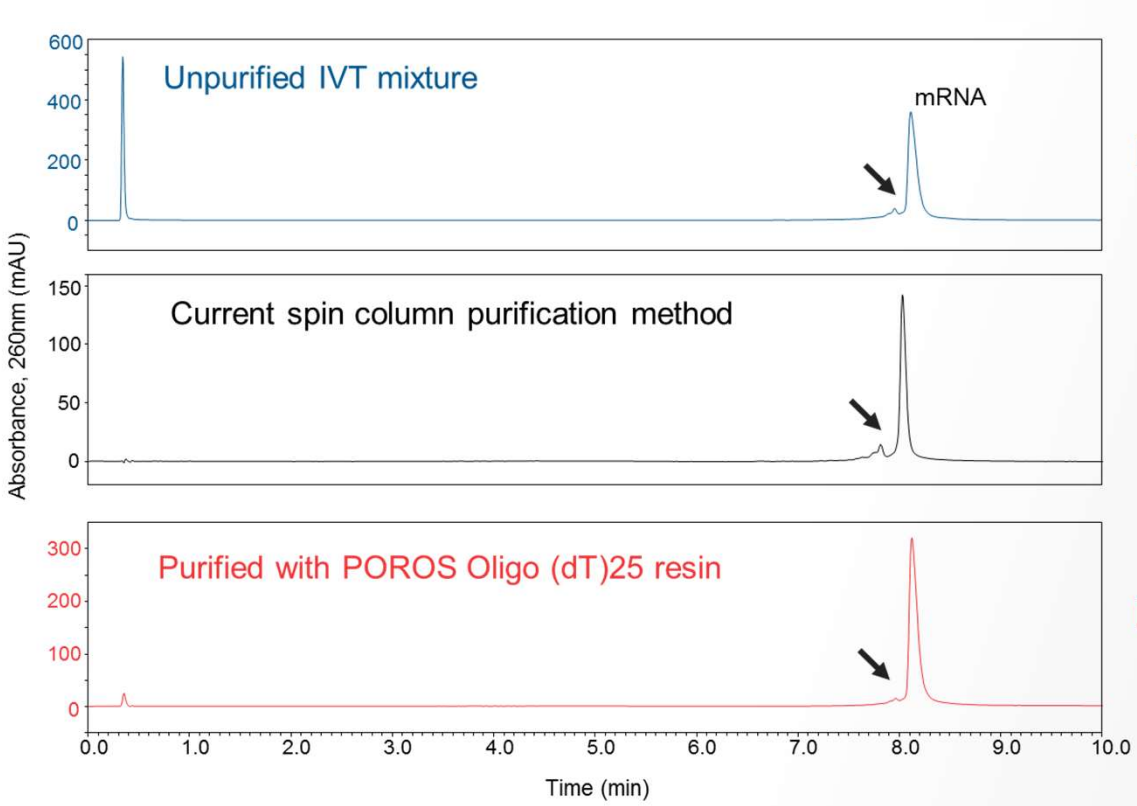
Samples provided by AmpTec Amplification Technologies

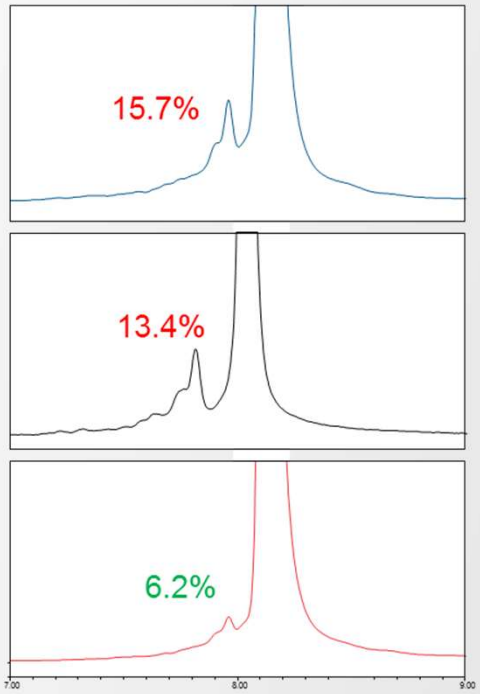


IVT mixture:
- ss-mRNA
- ds-mRNA
- DNA
- Enzymes

Efficient removal of impurities compared to the spin column method



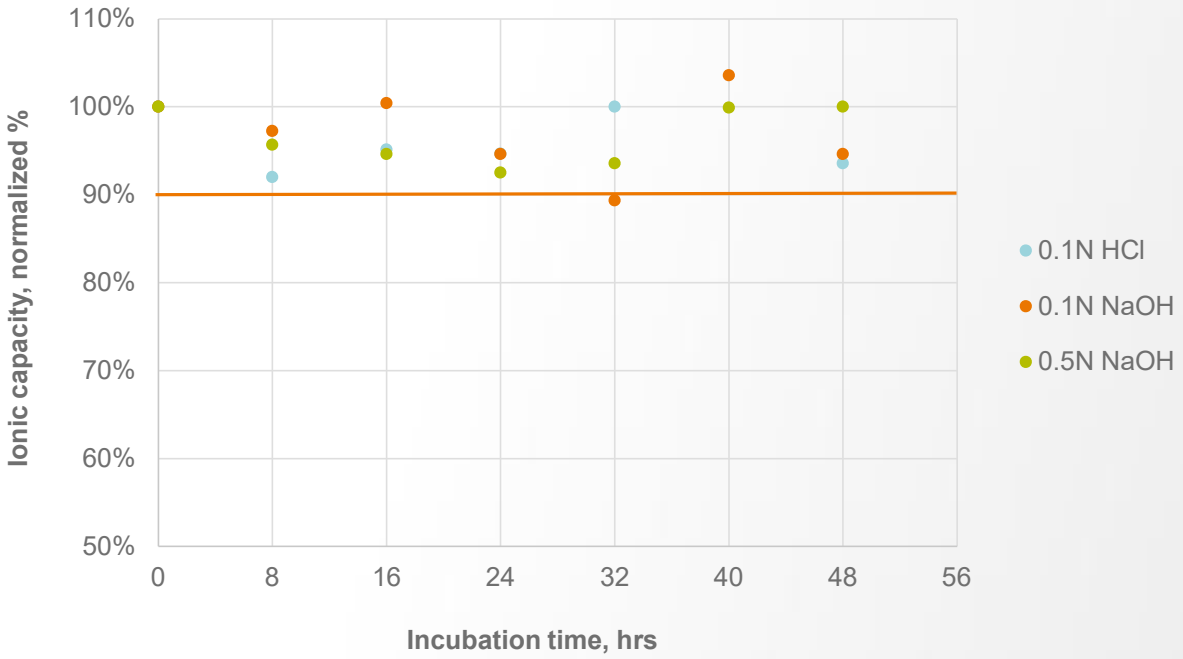
Improved impurity clearance



Samples provided by AmpTec Amplification Technologies

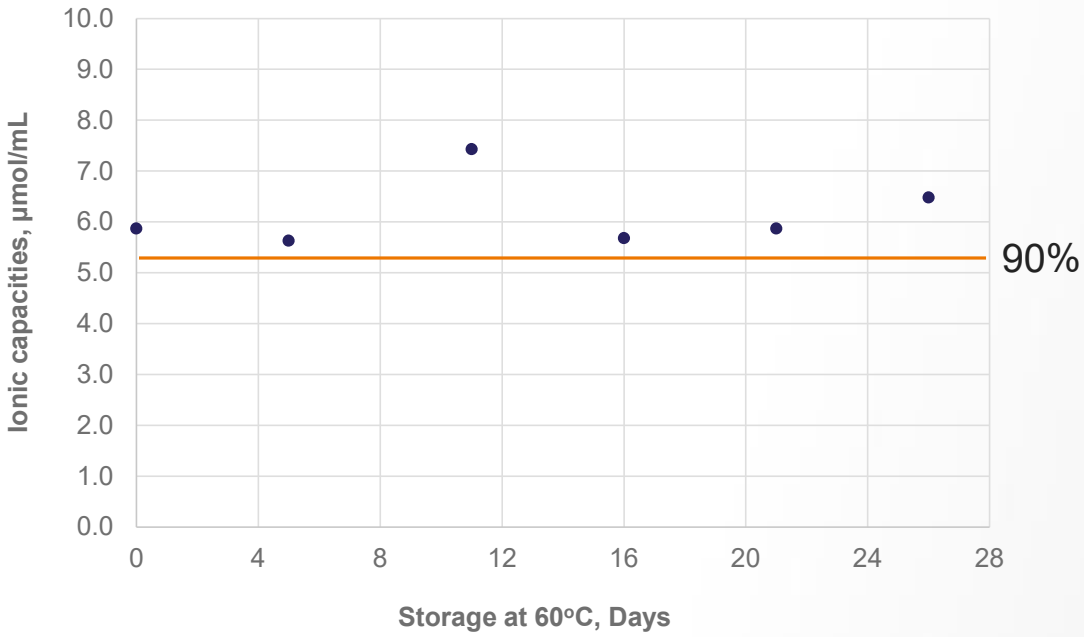
Purification with POROS Oligo (dT)25 leads to a significant reduction of impurities

Cleaning and stability of the Oligo (dT)25 affinity resin



The Oligo (dT)25 resin demonstrates good stability over a wide range of pH conditions (1-13) and can withstand 0.5N NaOH, allowing for stringent cleaning and sanitization

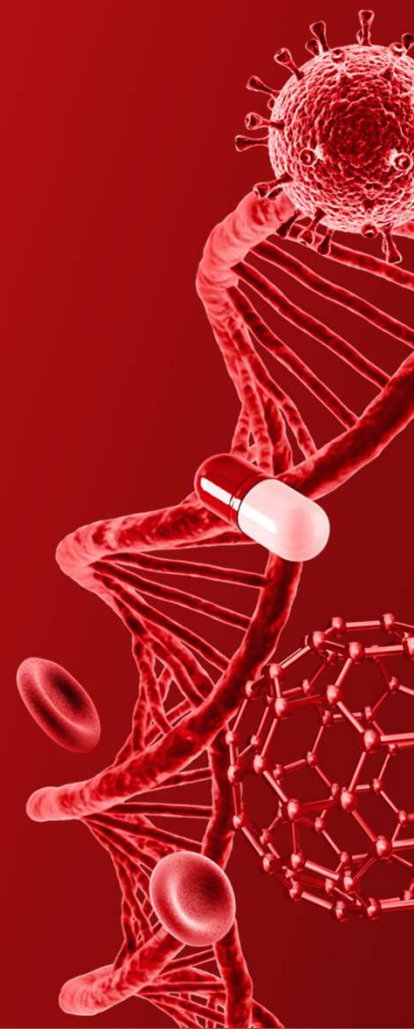
Accelerated stability of the Oligo (dT)25 affinity resin



Test storage condition	Predicted storage conditions	
Days at 60°C	Months at 25°C	Months at 5°C
5	6	66
11	12	138
16	18	204
21	24	276
26	30	342

Accelerated stability study demonstrates excellent predicted long-term stability of the resin

Thank you



mRNA Analytics: Product Characterization and Quality Monitoring

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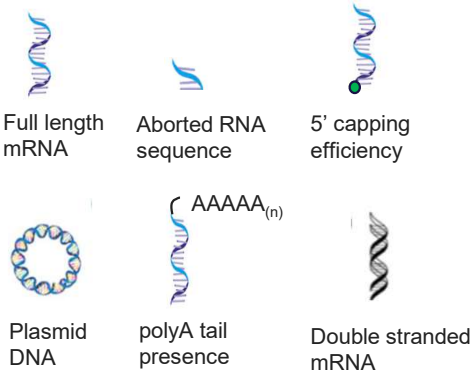
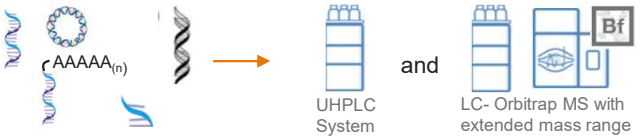
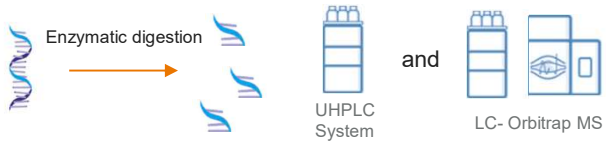
Purity/impurity analytics includes



Thermo Scientific™ mRNA analytics chromatography and mass spectrometry products of interest

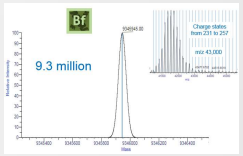
- Vanquish™ UHPLC Systems
- Orbitrap™-based mass spectrometers
- DNAPac™ RP column
- Acclaim™ 300 C18 HPLC column
- Accucore™ C18 HPLC column
- BioPharma Finder™ software

Purity/ Impurity	Workflow solution
Uncapped mRNA	
The presence of a 5' cap structure is essential for subsequent steps in the life cycle of mRNA in eukaryotic cells. Therefore, the capping efficiency must be determined and monitored throughout development.	 Columns • DNAPac RP • Acclaim 300
mRNA Impurity separation	
In order to maximize gene expression, the purity of mRNA must be determined, impurities characterized, and then monitored throughout the process. This includes aborted sequences, double stranded RNA, polyA tail variants, and residual plasmid DNA.	 Columns • DNAPac RP
Nucleic acid mapping	
The sequence confirmation of mRNA and identity of variants can be determined by mRNA sequence mapping, which is very similar to peptide mapping in protein characterization. The mRNA is digested with a nuclease enzyme, separated, then identified.	 Columns • DNAPac RP • Accucore C18

DNAPac RP columns

Thermo Scientific™ Vanquish™ Flex LC system

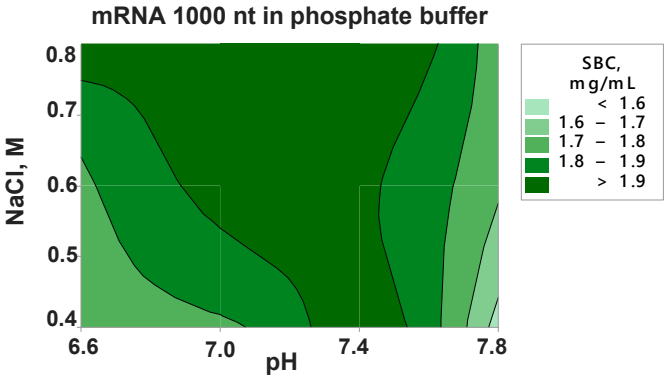
Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ mass spectrometer

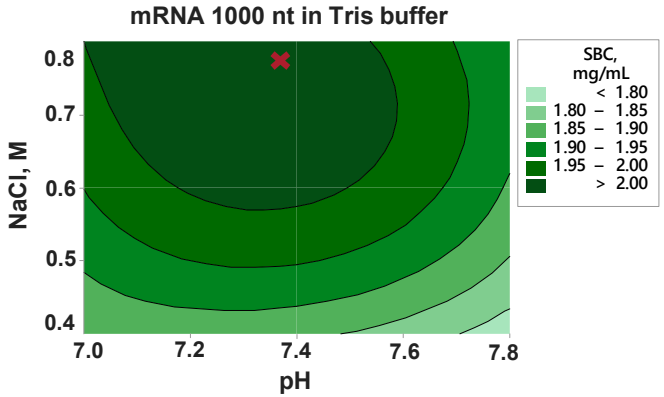


BioPharma Finder 4.0 software

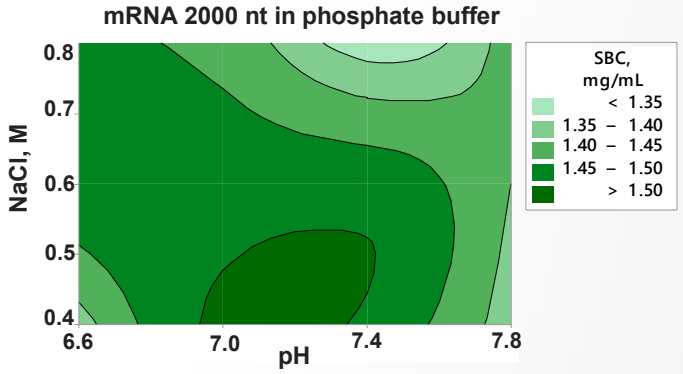
For Research Use Only. Not for use in diagnostic procedures.

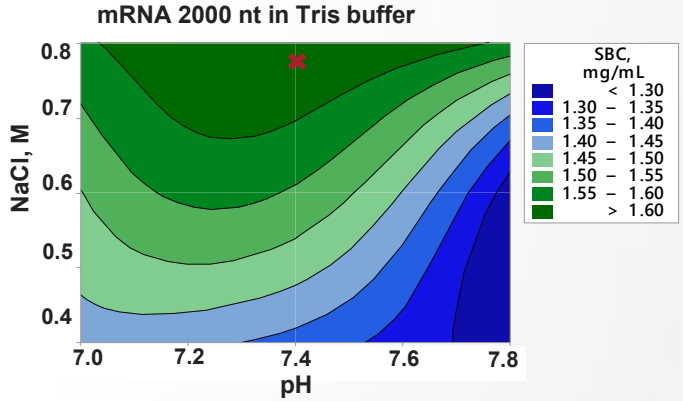
Finding the right binding buffer – Contour plots of static binding capacity





✖ 10mM Tris, 1mM EDTA, 0.8M NaCl, pH 7.4





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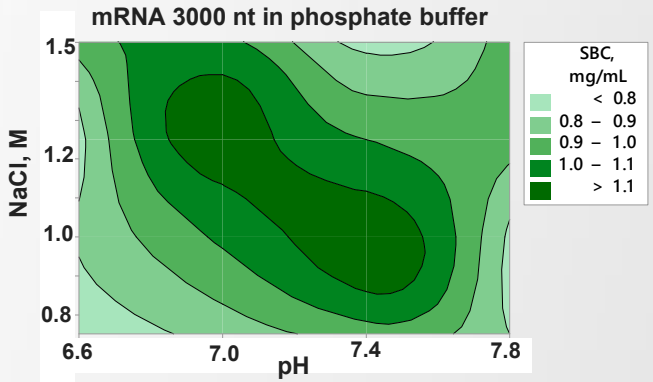

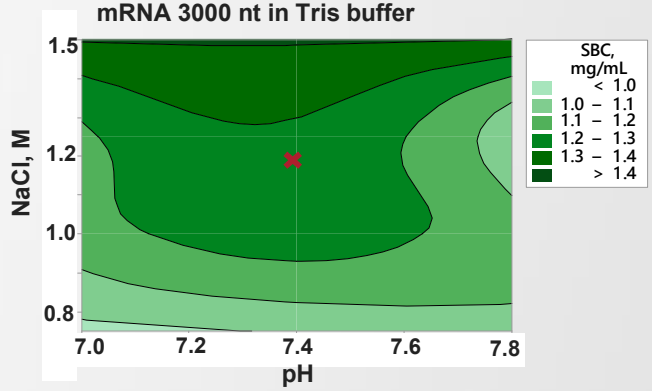

✖ 10mM Tris, 1mM EDTA, 1.2M NaCl, pH 7.4

Optimal binding conditions are construct dependent