

mRNA Purification and Analysis

mRNA HPLC Analysis on SEC, RP, Affinity, and IEX

Sepax Technologies, Inc.

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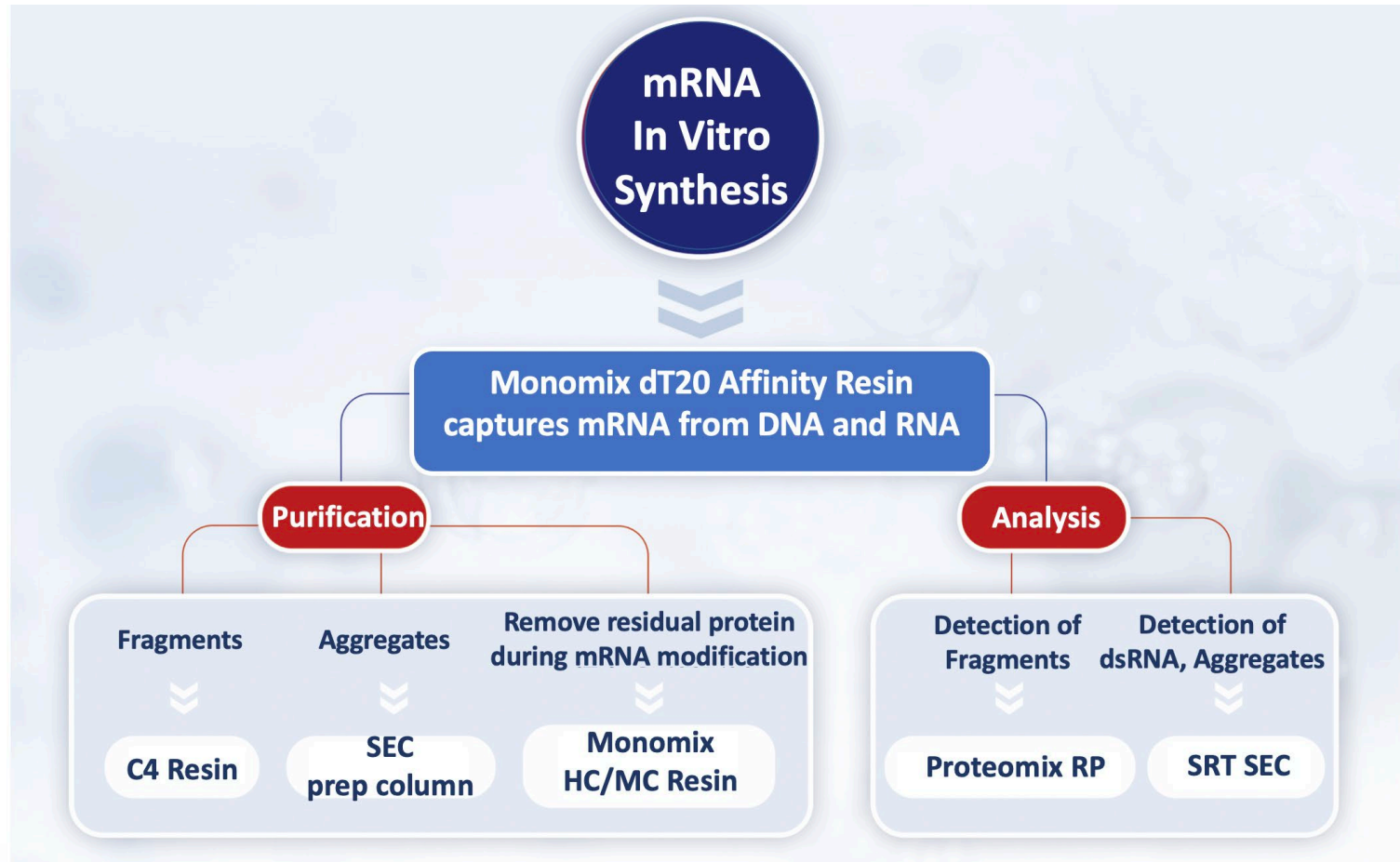
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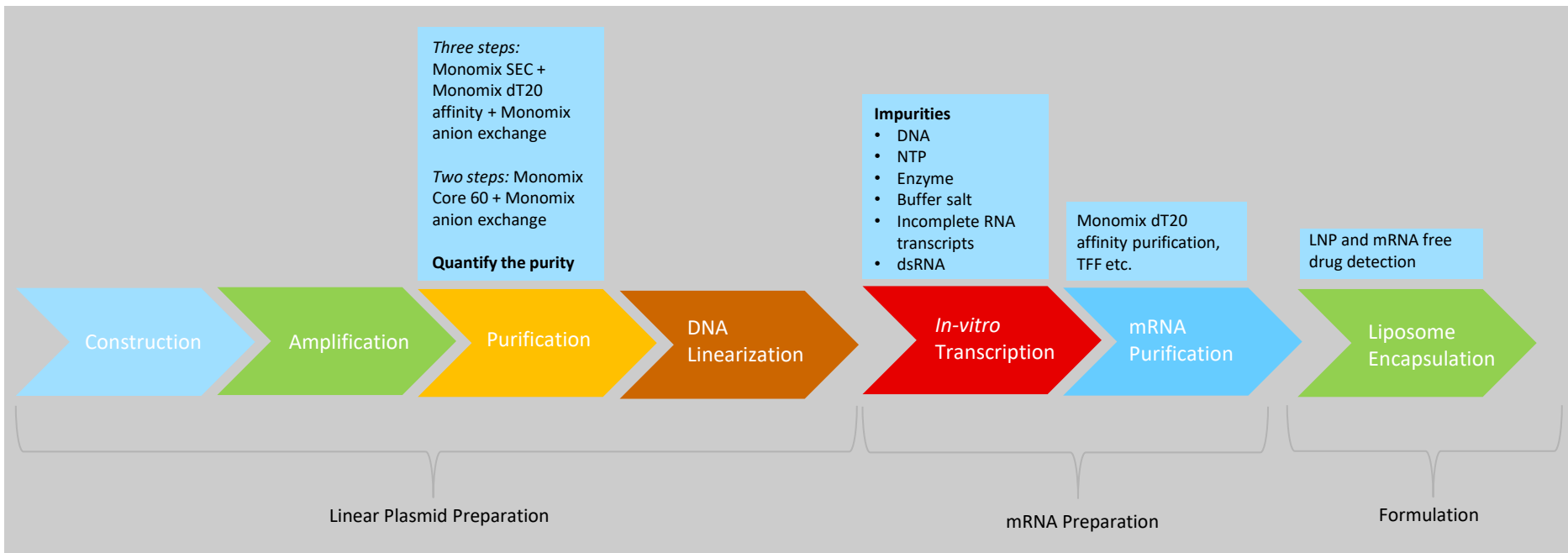
mRNA Chromatography Applications

The application of mRNA in the therapeutic and vaccine fields has led to a worldwide demand in chromatography technology for both analytical and manufacturing processes. mRNA drug and research development require robust and competent methods to evaluate mRNA integrity, which is essential for therapeutic effect and/or immunogenicity.



mRNA Production Process

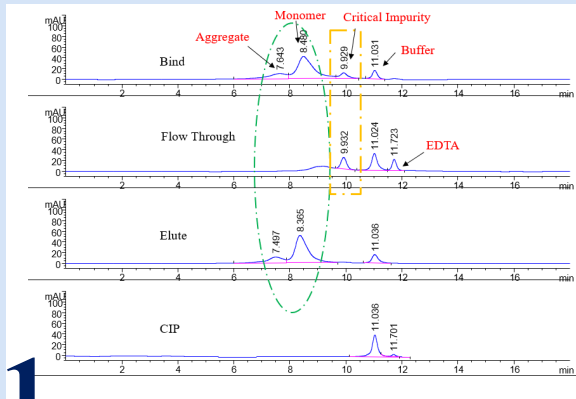
Example: Covid-19 mRNA Vaccine



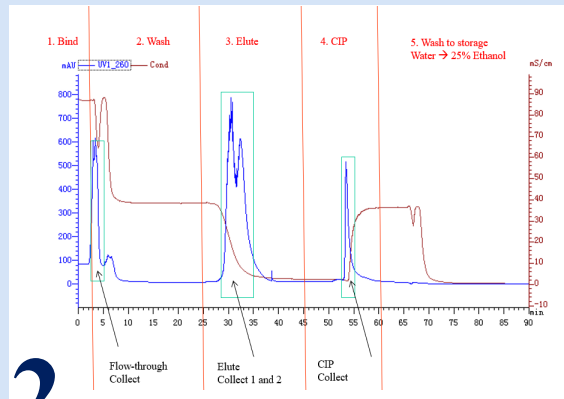
General Description

Sepax Monomix dT20 Affinity Resin is 30 μm narrowly dispersed polymethacrylate rigid base bead is functionalized with a polyhydroxylated surface coating layer that provides a bioinert surface and shows low non-specific binding. Resin surface of Monomix dT20 can capture mRNA through base pairing between oligo dT20-mer and the mRNA polyA tail, which can be utilized to simplify the subsequent purification steps and maximize overall production efficiency. After purification, quality consistency of purified mRNA was accessed by Size exclusion chromatography SEC method, here using Sepax SRT SEC-1000 column. Slide 1 shows the complete running sequence (including Bind, Wash, Elute, CIP and Storage stages) of a simplified purification process of mRNA using Monomix dT20 affinity resin. Slide 2 shows the stack SEC profiles of initial mRNA sample (a) and the fractions collected from Wash (b), Elute (c), and CIP (d) stages on SRT SEC-1000 column.

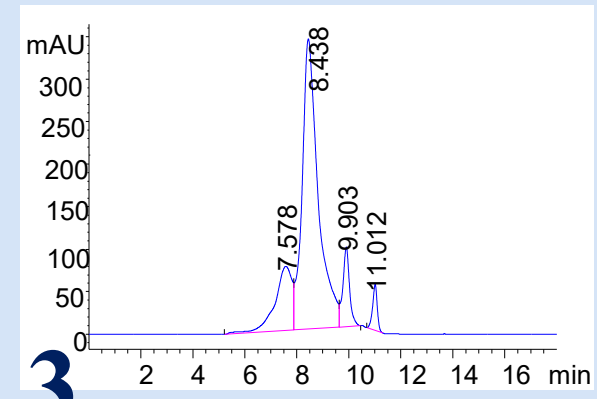
In mRNA therapeutic development, size exclusion chromatography SEC has been utilized for its quick reliable method in many applications such as purification or aggregate quantification. Slide 3 shows the application of separating single stranded mRNA monomer, its aggregates including possible double stranded mRNA and mononucleotides. Additionally, Reverse-phase (RP) chromatography can also be used as an orthogonal method for further characterization of each SEC peak.



1 Monomix dT20 affinity resin purification of mRNA sample



2 Overlay of mRNA fractions analysis



3 SRT SEC-1000 can separate mRNA monomers and aggregates



Sepax Monomix dT20 Affinity Resin



Part Number: 283030950-0000: 1 mL, 5 mL resin

Part Number: 283030950-750100: 4.2 mL cartridge

Part Number: 283030950P-2105: 2.1 x 50 mm PEEK

Part Number: 283030950P-4605: 4.6 x 50 mm PEEK

Shipping and Storage Solvent: 20% Ethanol with Water
Shipping Condition: Wet Ice



Characteristics

- Provides efficient capture and release under standard mRNA purification conditions, simplify subsequent purification steps and maximize overall production efficiency.
- Decreases process development time and enhances productivity.
- Remove plasmid DNA and other transcription components.
- Stable at elevated temperatures for the breakdown of undesired higher order structures, if needed.
- Excellent scalability. Provide prepacked columns, semi-prep columns, prep columns and bulk resin.
- Non-animal derived
- Resin customization. Polymethacrylate bead size is available at 10, 15, 30 and 60 μ m.



Technical Specifications

Resin Type	Monomix dT(20)
Base & Support Matrix	Hydrophilic polymethacrylate, Monomix 1000 Å
Particle Size D50 (µm)	30
Average Pore Size (Å)	1000
Functional Group	Oligo dT20-mer
Ligand density	≥2.1 mg dT20 / mL of resin
Binding Capacity	>2.0 mg mRNA per mL of resin
mRNA Binding Capacity (mg/ml resin)	≥2.0 mg
Max Linear Flow Rate (cm/hr)	1000
Operating Temperature (°C)	4 – 65
pH Stability	2 – 12
Operating Pressure	≤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, 2-25°C. Do not freeze resin or column.
CIP	0.1-0.5M NaOH. Recommend to start with 0.1M NaOH to prolong resin life



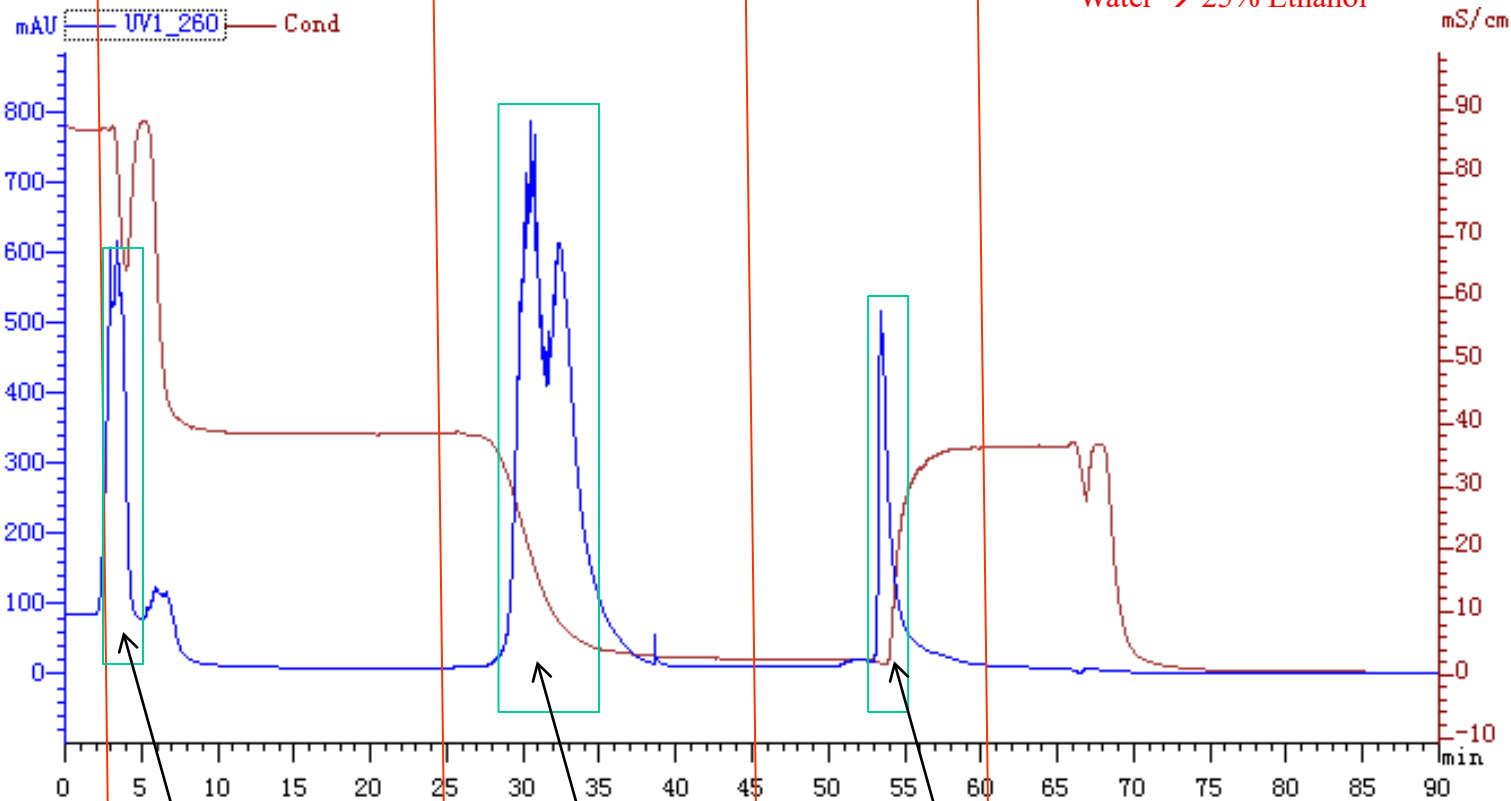
1. Bind

2. Wash

3. Elute

4. CIP

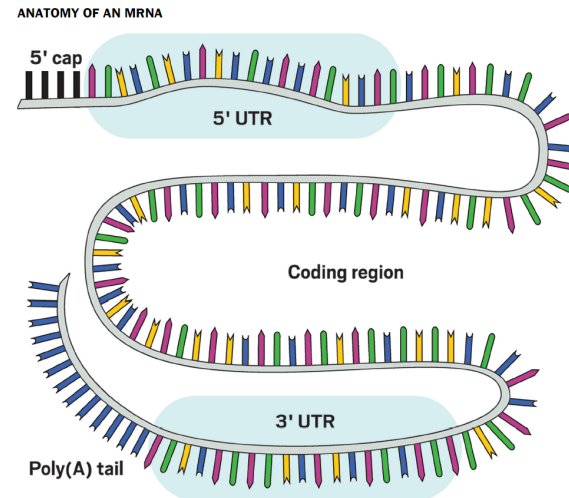
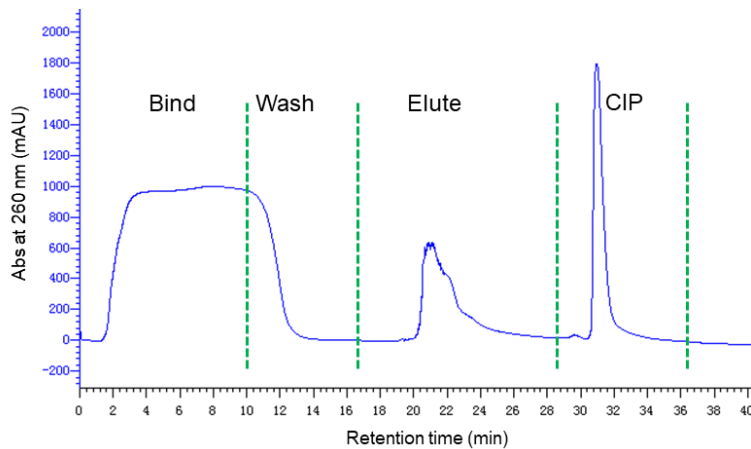
5. Wash to storage
Water → 25% Ethanol



Sepax Monomix dT20 Affinity Resin

COVID 19 mRNA vaccine candidate

Essential for mRNA commercial production, performance comparable to name brand

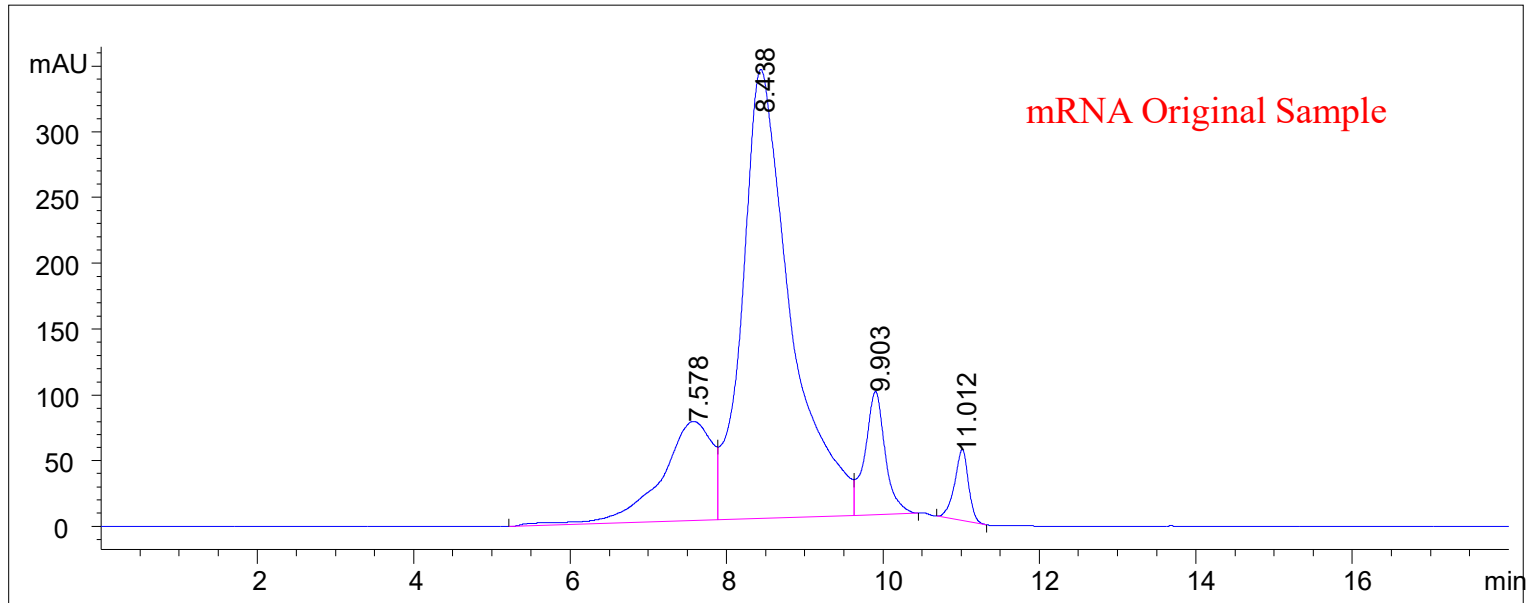


mRNA COVID-19 Vaccine Capture



mRNA Sample Analysis on SEC-1000

Column: SRT SEC-1000 5 μ m, 7.8 x 300 mm; Part Number: [215950-7830](#); Mobile Phase: 150mM PB pH7.0; Flow Rate: 1 mL/min; Detector: UV 260 nm; Column Temperature: RT; Injection Volume: 5 μ L; Sample: mRNA original sample (1mg/L); Pressure: 61bar; Instrument: HPLC

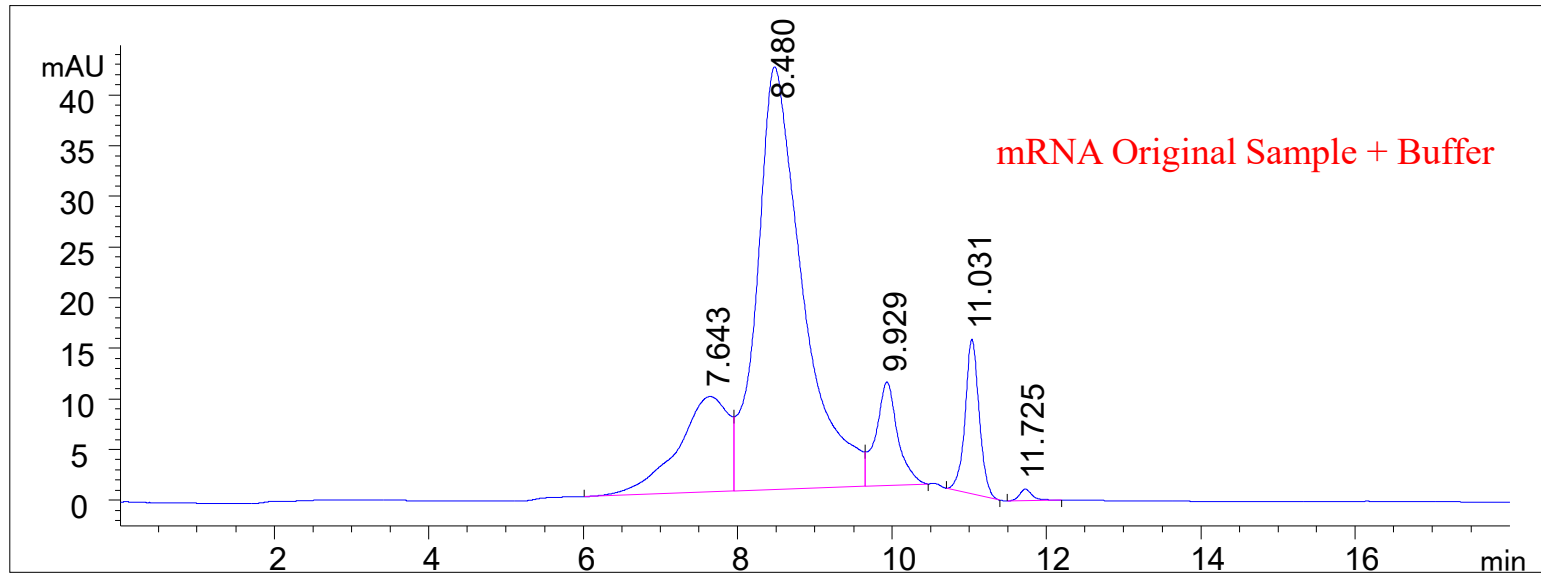


#	RT	Area	Height	Width	Tailing	Area%
1	7.578	3442.2	75.4	0.6659	1.965	16.826
2	8.438	14639.9	341.2	0.6416	0.579	71.562
3	9.903	1708	93.6	0.2658	1.034	8.349
4	11.012	667.6	54.2	0.1827	1.185	3.263



Sample + Buffer on SEC-1000

Column: SRT SEC-1000 5 μ m, 7.8 x 300 mm; Part Number: [215950-7830](#); Mobile Phase: 150mM PB pH7.0; Flow Rate: 1 mL/min; Detector: UV 260 nm; Column Temperature: RT; Injection Volume: 5 μ l; Sample: mRNA Original + Buffer (380ul dilute to 1ml); Pressure: 61bar

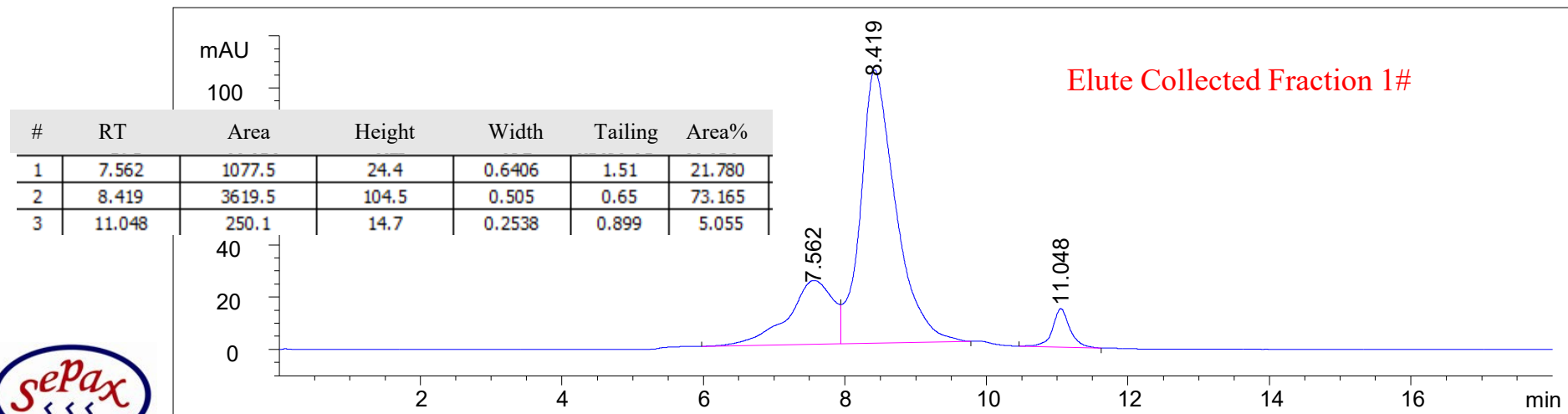
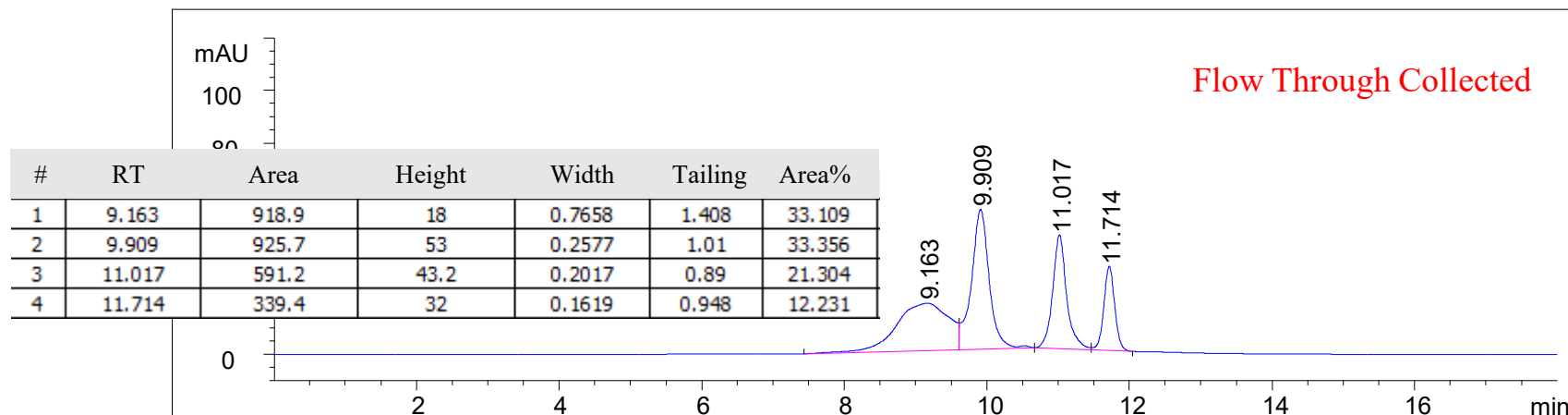


#	RT	Area	Height	Width	Tailing	Area%
1	7.643	427.5	9.4	0.6492	1.902	16.803
2	8.48	1723	41.7	0.5811	0.611	67.726
3	9.929	200.2	10.2	0.281	0.983	7.870
4	11.031	193.4	15.3	0.1899	0.902	7.601



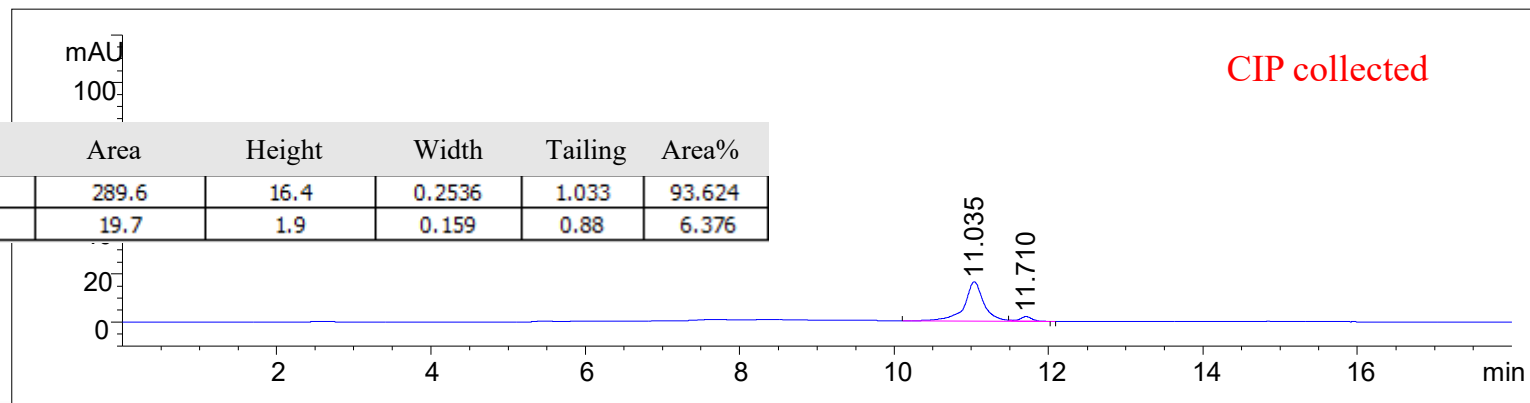
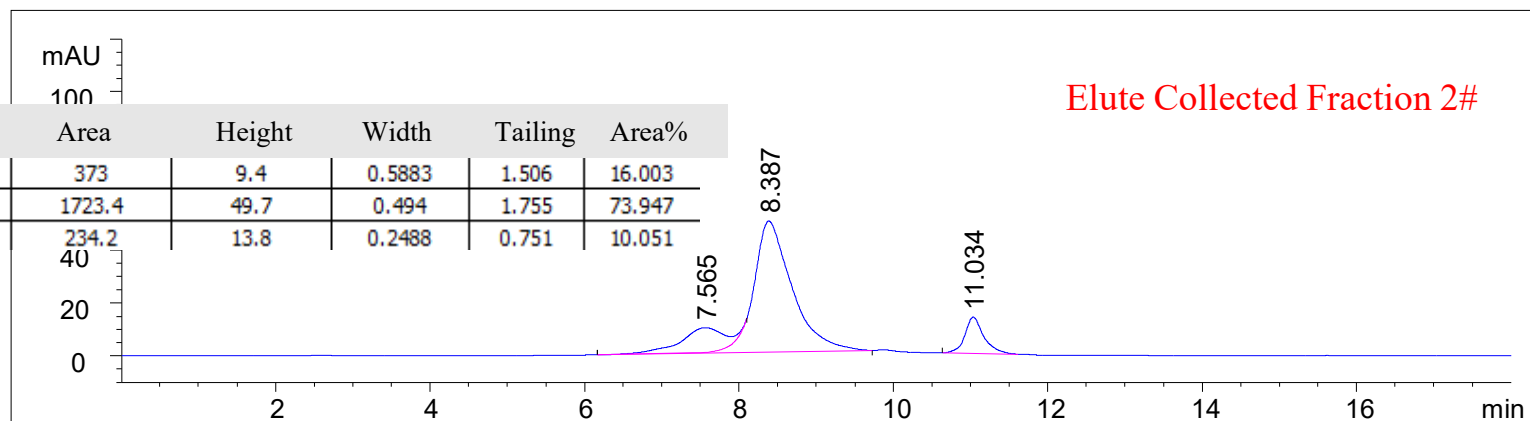
Collected Fractions Analysis on SEC-1000

Column: SRT SEC-1000 5µm, 7.8 x 300 mm; Part Number: [215950-7830](#); Mobile Phase: 150mM PB pH7.0; Flow Rate: 1 mL/min; Detector: UV 260 nm; Column temperature: RT; Injection Volume: 20µl; Sample: Collected Fractions; Pressure: 61bar



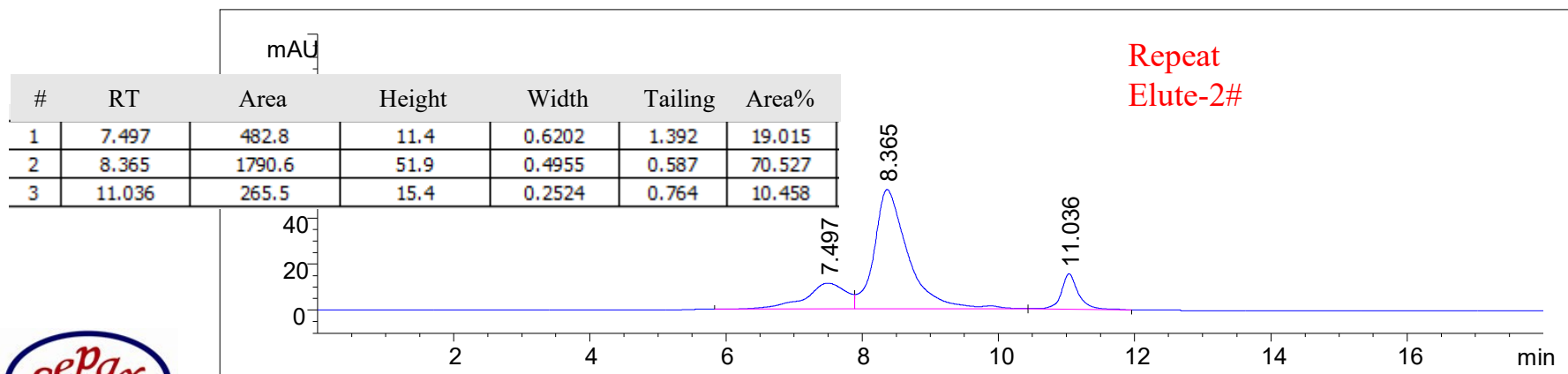
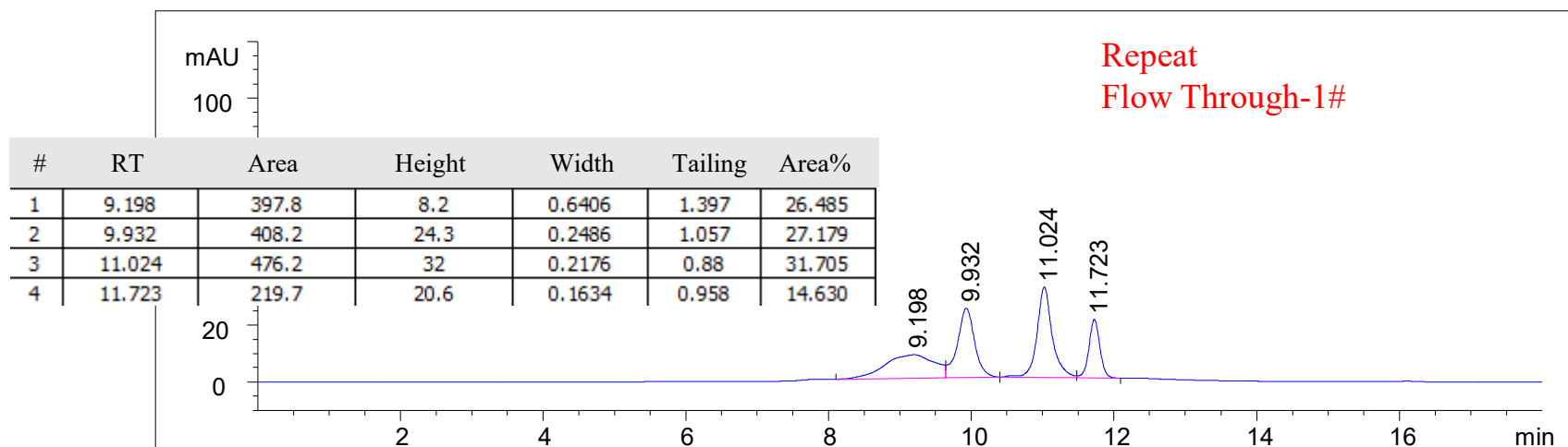
Collected Fractions Analysis on SEC-1000

Column: SRT SEC-1000 5 μ m, 7.8 x 300 mm; Part Number: [215950-7830](#); Mobile Phase: 150mM PB pH7.0; Flow Rate: 1 mL/min; Detector: UV 260 nm; Column Temperature: RT; Injection Volume: 20 μ l; Sample: Collected Fractions; Pressure: 61bar

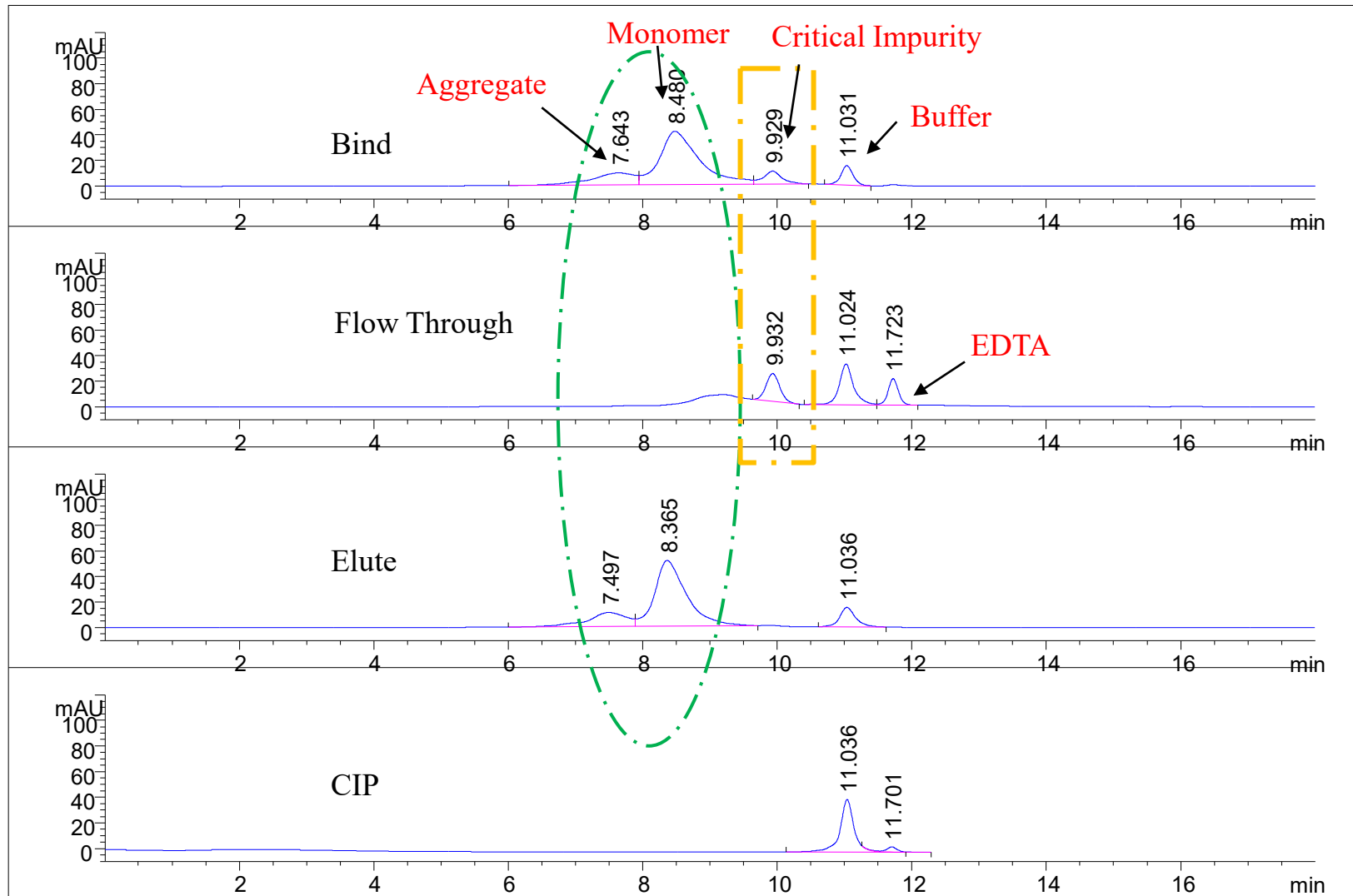


Collected Fractions Analysis on SEC-1000

Column: SRT SEC-1000 5 μm , 7.8 x 300 mm; Part Number: [215950-7830](#); Mobile Phase: 150mM PB pH7.0; Flow Rate: 1 mL/min; Detector: UV 260 nm; Column Temperature: RT
Injection Volume: 20 μl ; Sample: Collected Fractions; Pressure: 61bar

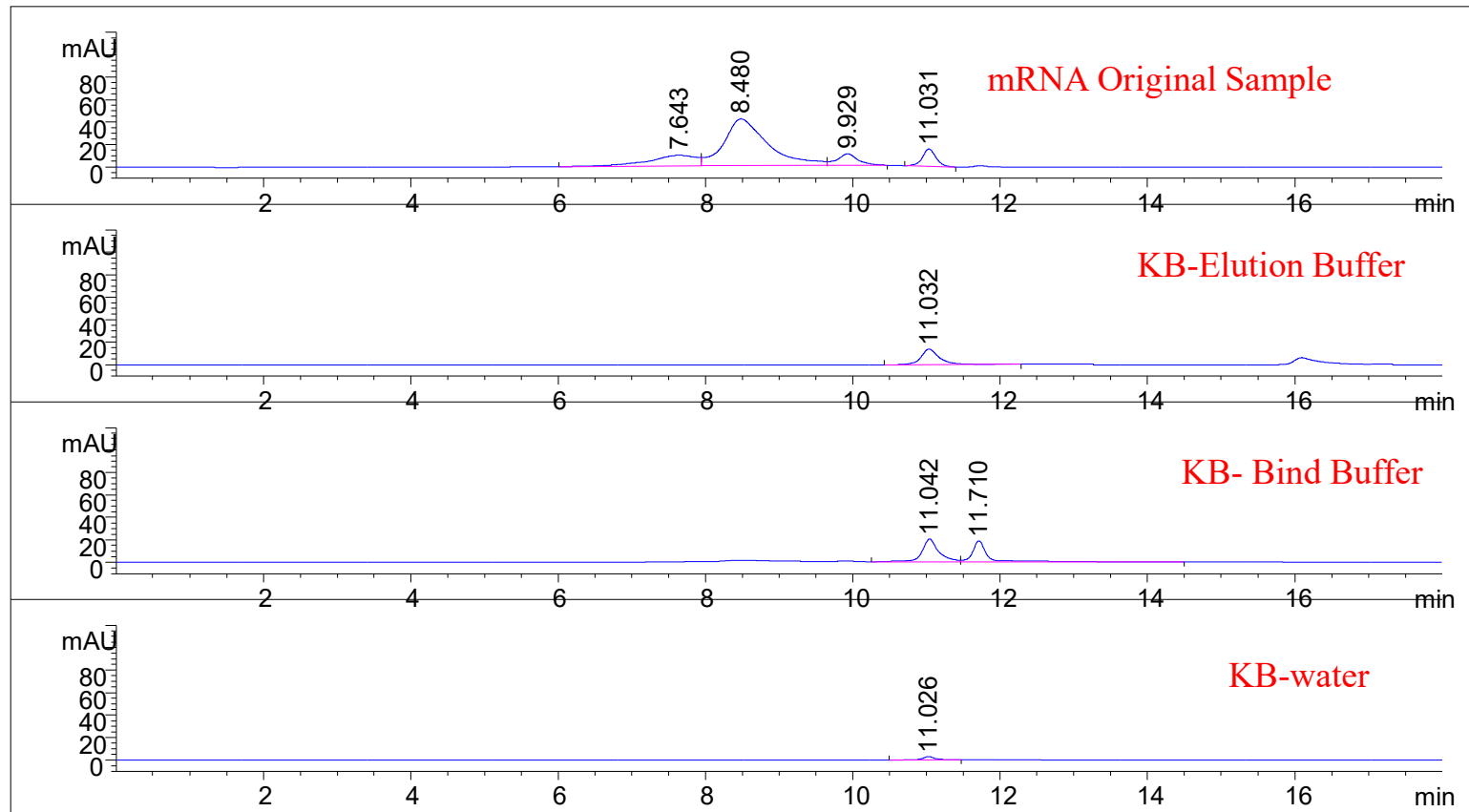


Fractions Overlays



Buffer Overlays

Column: SRT SEC-1000 5 μ m, 7.8 x 300 mm; Part Number: [215950-7830](#); Mobile Phase: 150mM PB pH7.0; Flow Rate: 1 mL/min; Detector: UV 260 nm; Column Temperature: RT
Injection Volume: 20 μ l; Sample: Blank; Pressure: 61 bar





SEPAX APPLICATION NOTE

mRNA Separation on SEC and RP

SRT SEC-1000, 5 μm , 1000 \AA , 7.8 x 300 mm

Part Number: 215950-7830

Proteomix RP-1000, 5 μm , 1000 \AA , 2.1 x 100 mm

Part Number: 465950-2110

Sample: mRNA (Single-stranded, 1000 nucleotides, 300K ~ 600K Da)

Concentration: 0.5 mg/mL

Sample solution: Water (DEPC)

Sample pH: Neutral

Storage: -20 °C

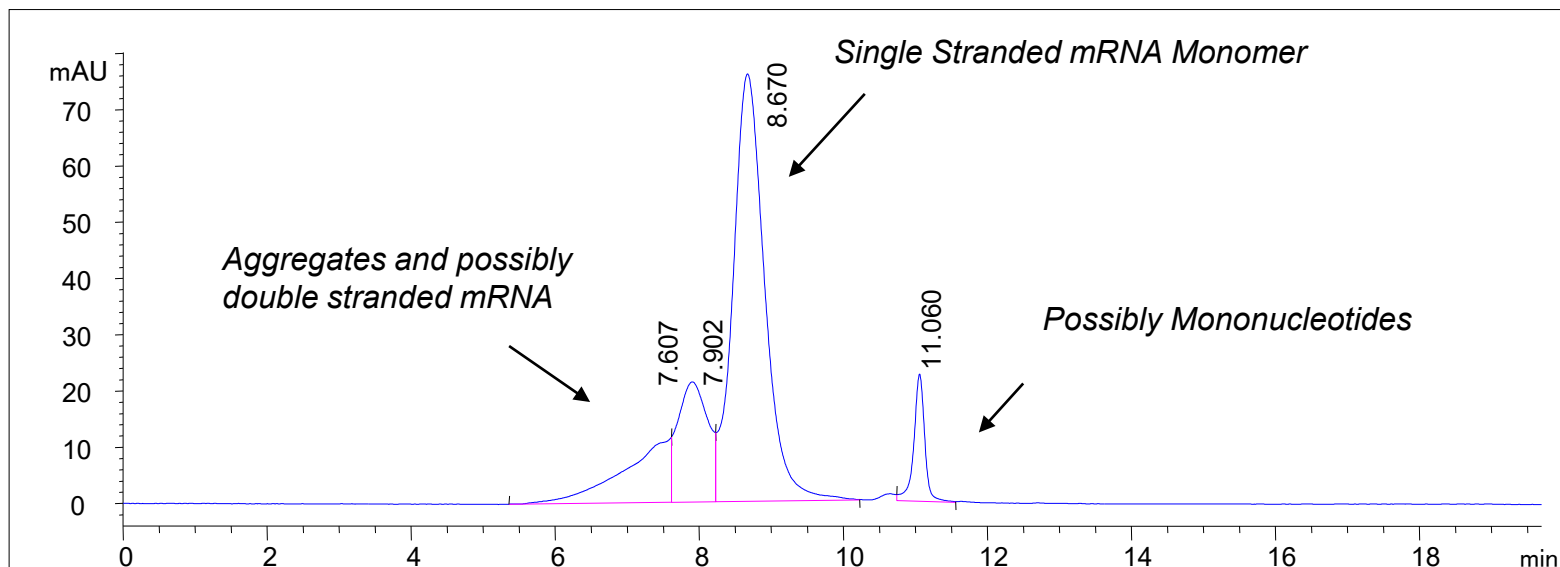
Impurity: Aggregates, fragments, double strand impurities



mRNA-1 on SRT SEC-1000

Column: SRT SEC-1000, 5 μm , 1000 \AA , 7.8 x 300 mm ([215950-7830](#)); Mobile Phase: 150 mM PB, pH 7.0; Flow Rate: 1.0 mL/min; Detector: UV 260 nm; Column Temperature: 25 $^{\circ}\text{C}$; Injection Volume: 5 μL , Sample: mRNA-1 0.5 mg/mL

RP can be used as orthogonal method for further characterization of each SEC peak.

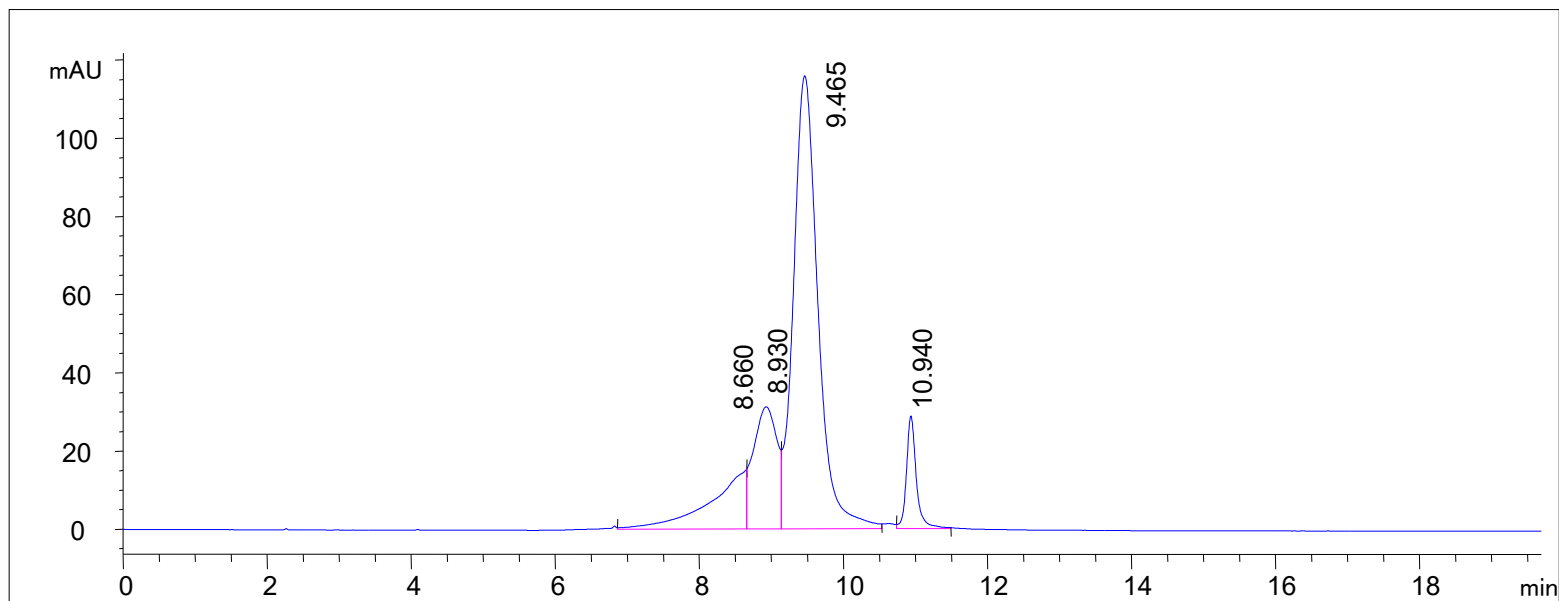


RT [min]	Height	Area	Area%	Plates	Tailing	Resolution
7.607	11	517.2	13.952	832	0.50	
7.902	21	623.2	16.812		-1.74	
8.670	76	2329.6	62.847	2055	1.23	
11.060	23	236.9	6.390	31503	0.78	4.71



mRNA-1 on SRT SEC-2000

Column: SRT SEC-2000, 5 μm , 1000 \AA , 7.8 x 300 mm ([215980-7830](#)); Mobile Phase: 150 mM PB, pH 7.0; Flow Rate: 1.0 mL/min; Detector: UV 260 nm; Column Temperature: 25 $^{\circ}\text{C}$; Injection Volume: 5 μL , Sample: mRNA-1 0.5 mg/mL

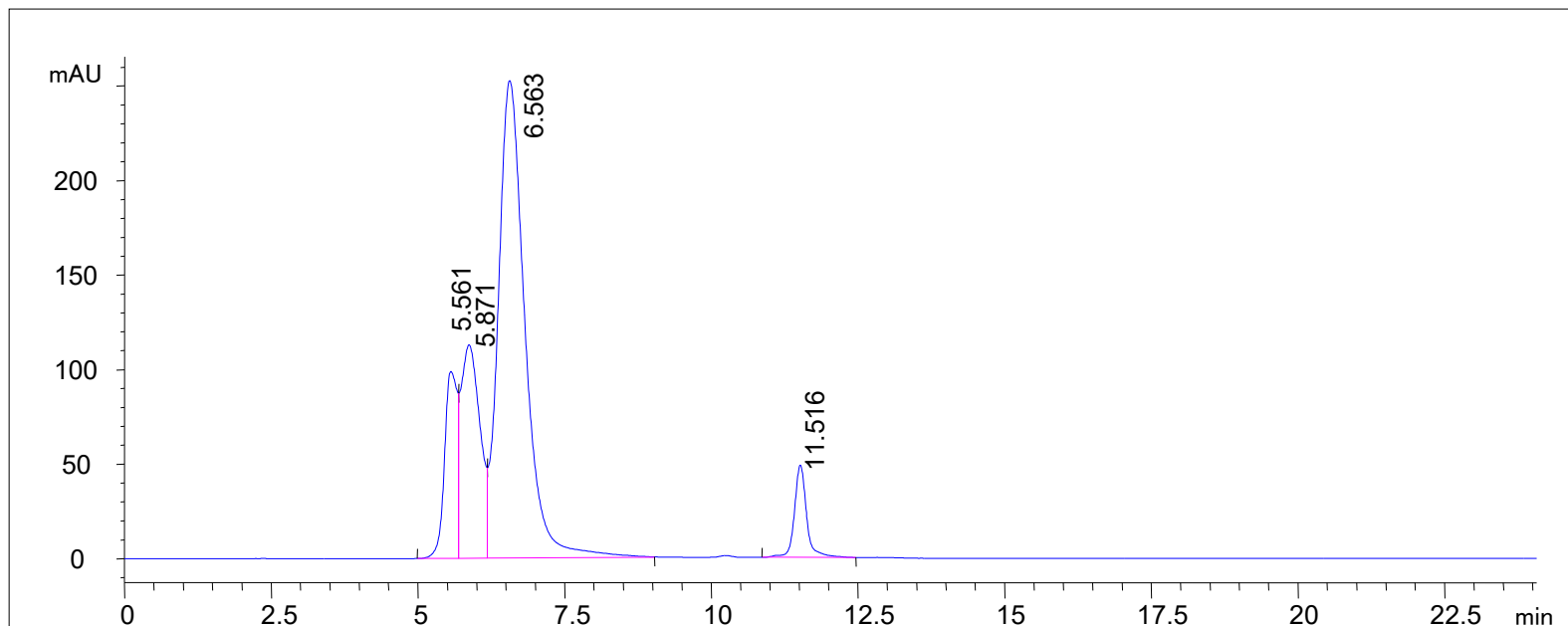


RT [min]	Height	Area	Area%	Plates	Tailing	Resolution
8.660	15	523.5	12.297	2397	0.50	
8.930	31	713.4	16.759	1967	-1.83	0.36
9.465	116	2745.1	64.487	4149	1.26	0.77
10.940	29	274.9	6.457	37511	1.22	3.62



mRNA-1 on SRT-C SEC-500

Column: SRT-C SEC-500, 5 μm , 500 \AA , 7.8 x 300 mm ([235500-7830](#)); Mobile Phase: 150 mM PB, pH 7.0; Flow Rate: 1.0 mL/min; Detector: UV 260 nm; Column Temperature: 25 $^{\circ}\text{C}$; Injection Volume: 5 μL , Sample: mRNA-1 0.5 mg/mL

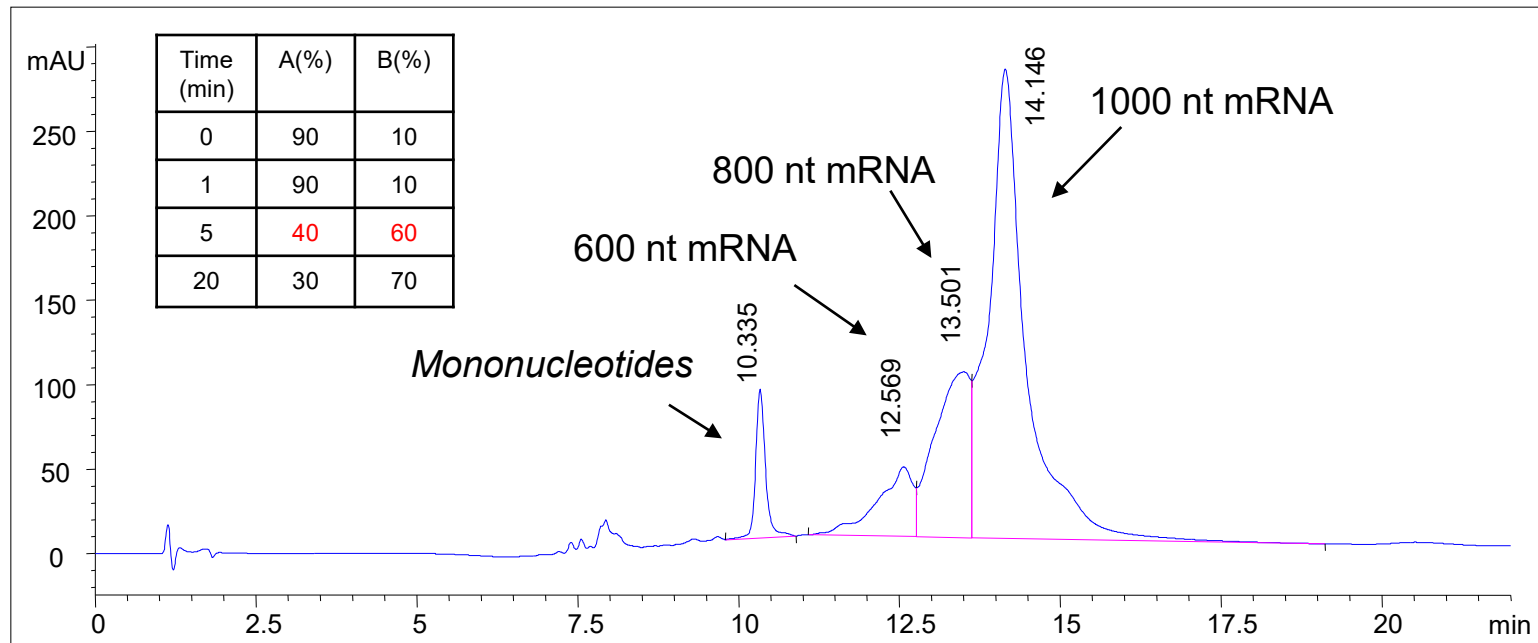


RT [min]	Height	Area	Area%	Plates	Tailing	Resolution
5.561	99	1498.5	11.772	2801	0.76	
5.871	113	2557.6	20.092	1101	-2.80	0.55
6.563	252	7959.4	62.527	1122	1.40	0.93
11.516	49	714.0	5.609	17461	1.25	8.74



mRNA-2 Analysis on Proteomix RP-1000

Column: Proteomix RP-1000, 5 μm , 1000 \AA , 2.1 x 100 mm ([465950-2110](#)); Mobile Phase: A: 100 mm TEAA B: 100 mm TEAA / 25% ACN; Flow Rate: 0.3 mL/min; Detector: UV 260 nm; Column Temperature: 50 $^{\circ}\text{C}$; Injection Volume: 10 μl ; Sample: mRNA-2; Pressure: 95 bar



Compound Name	RT [min]	Height	Area	Area%	Plates	Tailing	Resolution
	10.335	88.1	940.2	5.32	25906	1.30	
	12.569	41.4	1590.2	9.00	2275	0.58	3.40
	13.501	98.2	3762.6	21.30	2173	-0.68	0.84
	14.146	278.1	11371.9	64.38	4812	1.70	0.65



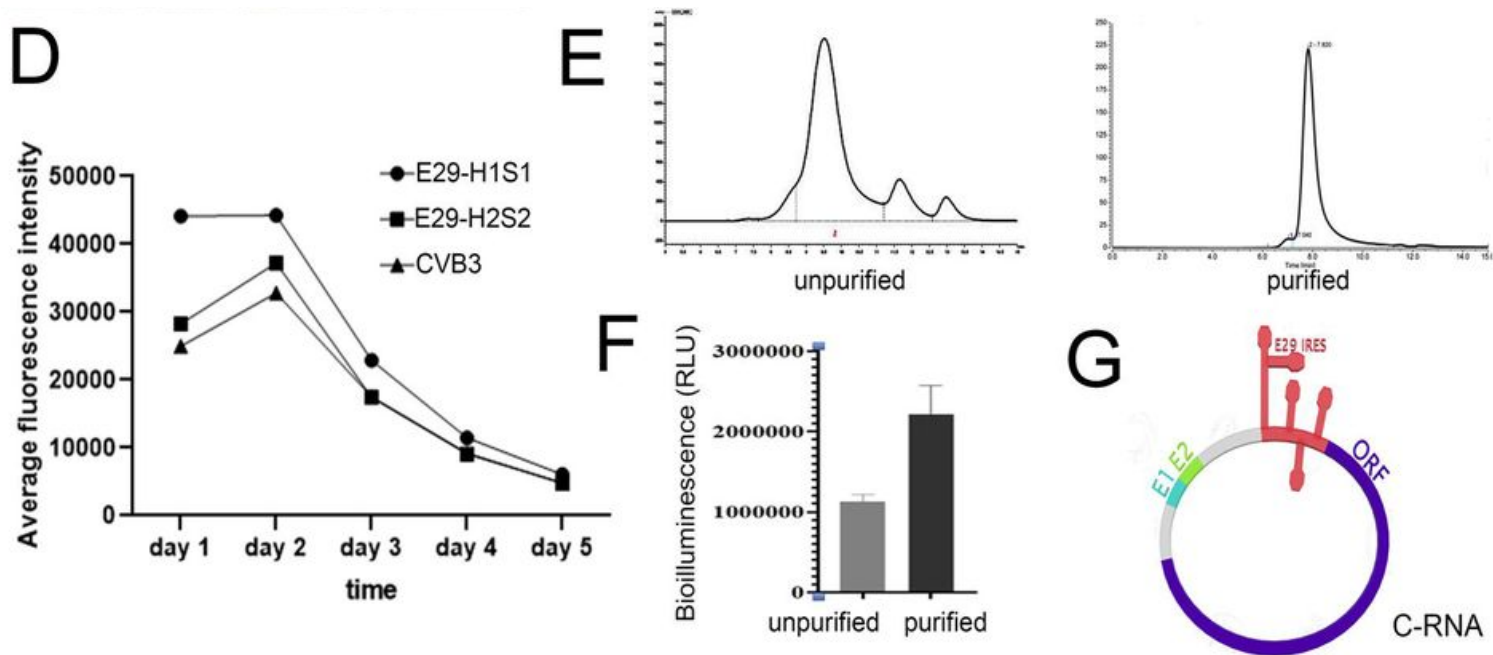
SEPAX LITERATURE REFERENCE

Circular mRNA Purification on SRT SEC 1000

Intratumoral Delivered Novel Circular mRNA
Encoding Cytokines for Immune Modulation and Cancer Therapy

Circular mRNA purification on SRT SEC 1000

Intratumoral Delivered Novel Circular mRNA Encoding Cytokines for Immune Modulation and Cancer Therapy



(D) Quantification of Fig.1C by FACS; (E) Diagram of HPLC shows the elution of circular mRNA before (left) and after (right) HPLC-SEC purifications; (F) Luciferase activities were measured 24 h post transfection of C-RNA with or without HPLC-SEC purifications; (G) Scheme of C-RNA.

Sepax PREP LC Column, SRT SEC-1000, 5 μ m, 1000 A, 30 x 300 mm

Part Number: [215950-30030](#)



Curemed Biopharma Technology

Yang, Jiali, et al. "Intratumoral Delivered Novel Circular mRNA Encoding Cytokines for Immune Modulation and Cancer Therapy." bioRxiv (2021).

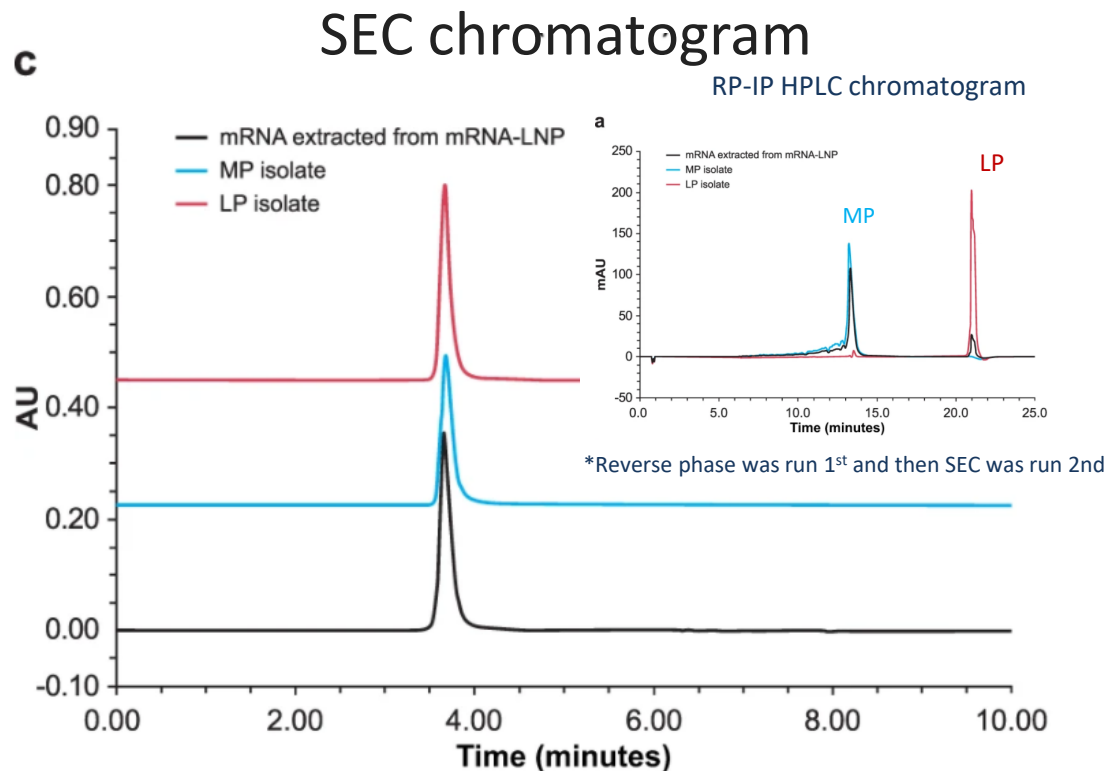
<https://doi.org/10.1101/2021.11.01.466725>

Literature Reference:

mRNA-LNP (Lipid Nanoparticle) on Sepax Analytical SEC

A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems

Column	Zenix SEC-300 4.6x 150mm Part Number: 213300-4615
Mobile Phase	100mM Tris acetate/2.5mM EDTA pH 8
Flow Rate/Detection	0.25 mL/min, UV 260nm
Instrument	Waters H-Class UPLC
Sample Notes	mRNA extracted from formulated mRNA-LNP
Length of Sample	~2500-3000 nucleotides
mRNA: Lipid prep	mRNA was extracted from the mRNA-LNP formulation by IPA precipitation. (IPA and then NH4-Acetate) Dry-vacuo and then resuspended in RNase-free H2O
MP and LP prep	RP-IP HPLC on extracted mRNA from LNP's and fractionated. Generating purified MP and LP fractions MP and LP fractions re-injected onto RP-IP HPLC and SEC



SEC was used to rule out if tertiary mRNA structures (i.e. aggregates) were the cause of the Late Peak

The SEC profile of the extracted mRNA vs. MP (main peak) vs. LP (late peak) were identical, thus eliminating aggregation as the origin of the late peak. This implicates other chemical reactions occurring to cause the generation of the late peak.



ModeRNA Therapeutics

Packer, Meredith, et al. "A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems." *Nature communications* 12.1 (2021): 1-11.

Order Information

SRT SEC-1000, 5 μm , 1000 \AA , 7.8 x 300 mm

Part Number: 215950-7830

Proteomix RP-1000, 5 μm , 1000 \AA , 2.1 x 100 mm

Part Number: 465950-2110

Monomix dT20 Affinity Resin 1 mL, 5 mL resin

Part Number: 283030950-0000

Monomix dT20 Affinity Resin 4.2 mL cartridge

Part Number: 283030950-750100

Monomix dT20 Affinity 2.1 x 50 mm PEEK

Part Number: 283030950P-2105

Monomix dT20 Affinity 4.6 x 50 mm PEEK

Part Number: 283030950P-4605

Resins and Columns are available for all your purification needs as well.

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Phone: 1-877-SEPAX-US

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