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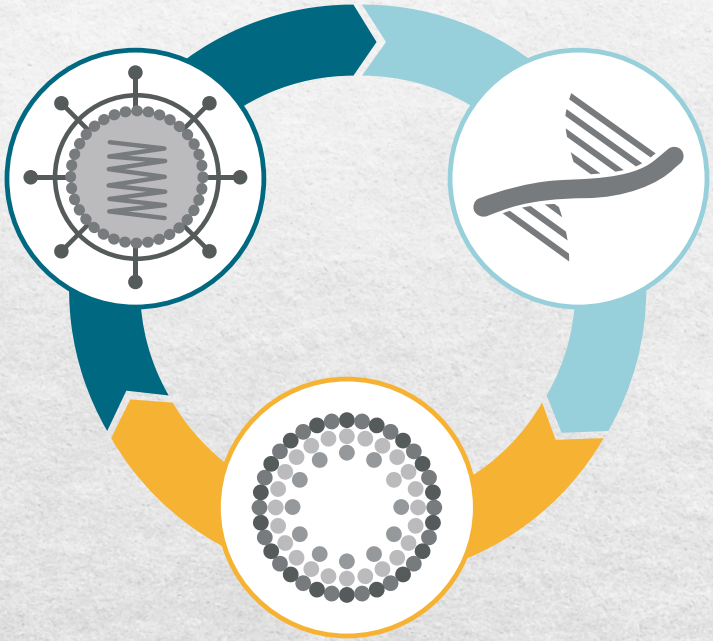
ThermoFisher SCIENTIFIC



LC columns

Analytical workflow solutions

to advance your **gene**, **mRNA**, and **oligonucleotide based biotherapeutics** process development

thermoscientific

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Introduction

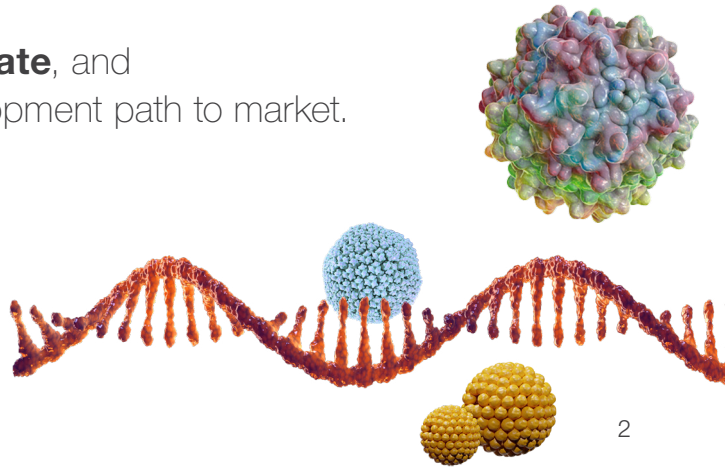
Advances in genetics and bioengineering have enabled the development of biotherapeutics with **unprecedented potential to treat diseases** caused by recessive gene disorders, acquired genetic diseases, and some viral infections.

Technologies for manufacturing and analysis of **gene**, **mRNA**, and **oligonucleotide based biotherapeutics** such as chromatographic methods are evolving with continued efforts to be fully optimized.

With the constantly growing number of clinical trials, the demand for biotherapeutics has increased significantly and the need for reliable process development and scalable manufacturing solutions has emerged.

In this workflow brochure we will discuss several process applications that are important for biotherapeutics manufacturers, including the strategies for choosing columns and accessories, and recommended workflows, from sample to results, to ensure that you get a great start with your new method.

Our robust solutions are designed to enable **fast, accurate**, and **reproducible results** to accelerate your product development path to market.



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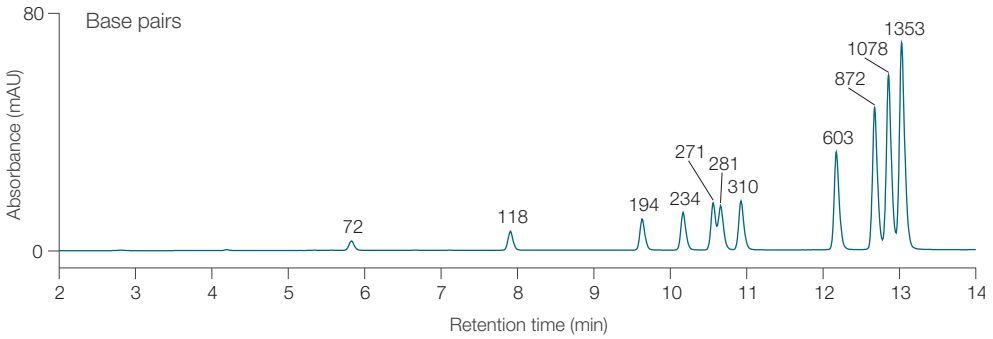
Chromatography as the method of choice

High-performance liquid chromatography (HPLC) is the method of choice if high-purity products are desired. This technique is an ideal fit in a scalable manufacturing workstream as it's less labor-intensive and faster than gel electrophoresis (10-minute runs vs 3-hour gel run) and offers possibility to automate.



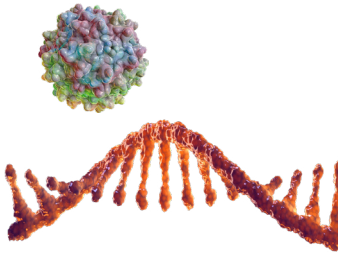


Figure 1: Separation of restriction enzyme digest. ΦX174-BsuRI digest (100 µg/mL).

Read this application note to learn more.

The Thermo Scientific™ Vanquish™ Flex UHPLC System is suitable for high resolution bioseparations and accurate measurements of biopharmaceutical monitoring with peak performance without any preconditioning or other preparation of the instrument. It is equipped with biocompatible features that are specifically selected materials that prevent undesired secondary interactions with the sample which can cause unreliable results. Its impeccable durability requires very little downtime compared to other instruments on the market – by providing you the space and time you need to run your analysis reliably every day.

Thermo Scientific instruments workflow solutions

Description	Quantity	Cat. no
Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC System	Each	IQLAAAGABHFAPUMBHV
Thermo Scientific™ Vanquish™ System Base	Each	VF-S01-A
Thermo Scientific™ Vanquish™ Quaternary Pump F	Each	VF-P20-A
Thermo Scientific™ Vanquish™ Column Compartment H	Each	VH-C10-A
Thermo Scientific™ Vanquish™ Split Sampler FT with 25 µL sample loop	Each	VF-A10-A
Thermo Scientific™ DNAPac™ RP Column	Each	088923

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Understanding the impact of vials on your analysis

Vials have more of an impact on biotherapeutic analysis than you would think, and therefore, selecting the right vial is important.

Hydrophobic proteins and **peptides** tend to create salt adducts with glass vials over time, reducing the recovery of certain proteins or peptides. Thermo Scientific™ SureSTART™ GOLD-Grade Vials have an ultra-low adsorption glass surface that enables trace-level analysis for strongly adsorbing analytes like proteins. These vials deliver the highest recovery rates with trisubstituted N-atoms and tertiary amines, as well as the lowest levels of alkaline materials for less glass-wall interactions.

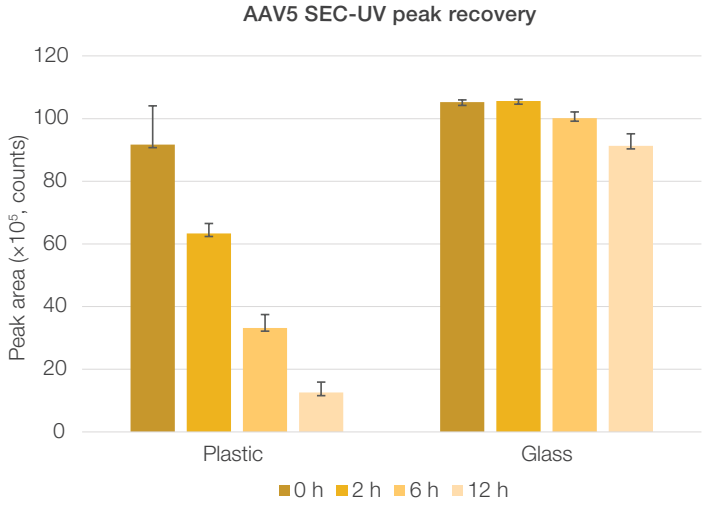
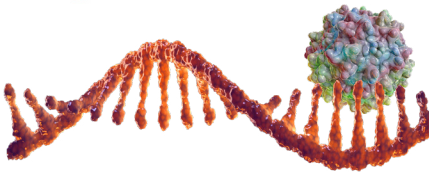


Figure 2. Bar graph plot of the peak area obtained after SEC-FLR analysis of AAV sample stored in plastic or glass vials at different time points

Oligonucleotides tend to form adducts with residual metal from the glass causing loss of recovery and robustness over time. To ensure the robustness of your method, Thermo Scientific™ SureSTART™ Polypropylene Vials should be used for oligonucleotide analysis.

For non-oligo biotherapeutics products, it's important to not only achieve the best sensitivity, but also assure robustness for small injection volumes. Thermo Scientific™ SureSTART™ High Recovery Glass Snap Top Microvials maximize injection volumes when analyzing <2 mL samples, and with their inner V-shaped bottom, enable a residual volume of 4 µL. When you have low sample volume, such as for lipid nanoparticles (LNPs) drug delivery vehicles, these vials are designed to protect your most valuable samples and deliver consistent, reproducible results for your most demanding analyses.



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Gene therapy workflow

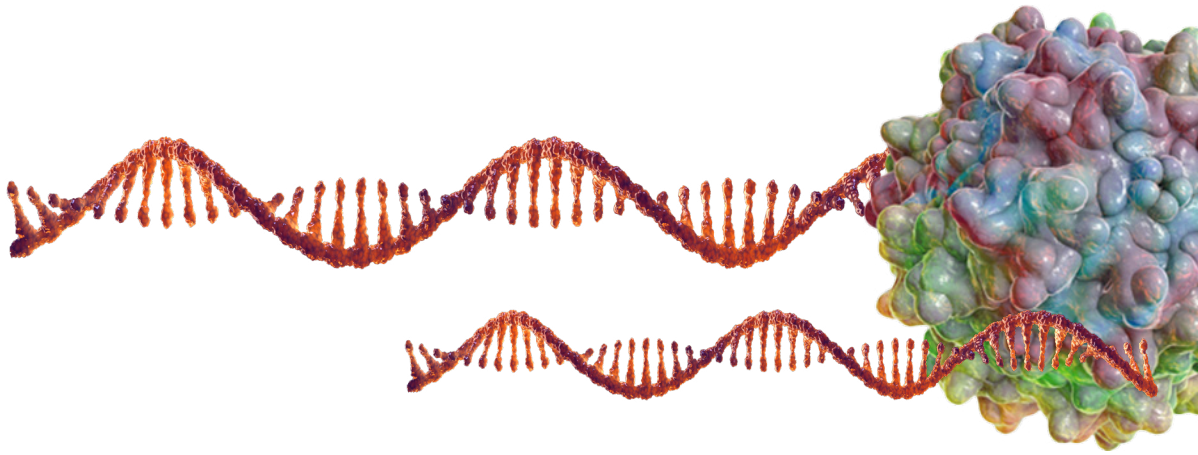
Introduction

Gene therapy seeks to modify or manipulate the expression of a gene to treat or cure disease by fixing the underlying cause. Gene therapy holds great promise for a wide range of diseases, including cancers, cardiovascular diseases and thousands of rare hereditary diseases caused by gene mutations as these mutations can be remedied by adding a functional copy of a gene, disabling a gene that makes a faulty product or changing gene activation.

The DNA genetic material that's delivered has instructions to change how a protein—or group of proteins—is produced by the cell. For some diseases, this means making changes to account for too much, not enough, or incorrect essential proteins being produced within cells.

This new genetic material, such as a working gene, is delivered into the cell:

- As plasmid DNA that can be encapsulated in a lipid nanoparticle to improve transfer into human cells or,
- Using viral vectors derived from viruses modified to remove disease-causing genes and replacing them with the gene(s) being transferred, and have a natural ability to deliver genetic material into human cells



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Gene therapy workflow

Plasmid DNA (pDNA) for gene therapy

In gene therapy manufacturing, high-quality pDNA is a key component. While non-viral, vector-like, naked pDNA is considered much safer, it's also less effective. The demand of high-quality plasmid DNA has increased, resulting in the need to optimize the manufacturing and the quality required for use in the manufacture of therapeutics. During plasmid production, supercoiled and other forms of product-related plasmid impurities are formed. Separation and quantification of these different pDNA forms from RNA and proteins define the purity of the plasmid sample.

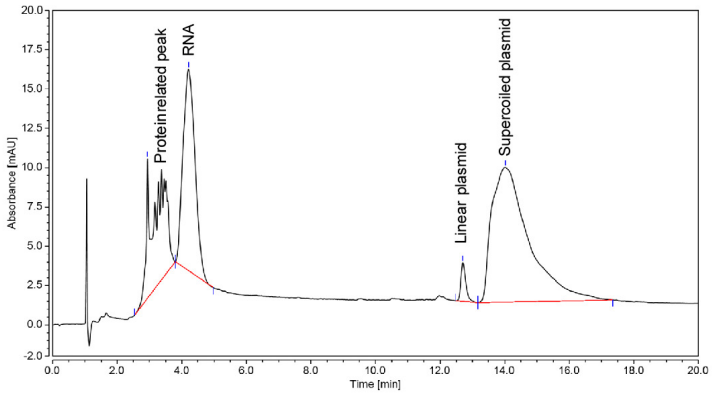

Figure 3. Separation of plasmid DNA on an anion exchange column. Chromatogram showing separation of different form of plasmid in presence of protein and RNA. Read this **application note** to learn more.

The columns

Thermo Scientific™ DNAPac™ PA200 Columns are a modern pellicular resin format that uses surface anion exchange sites developed for controlled resolution of single-stranded DNA (ssDNA) and RNA molecules. The surface exchange not only allows for high resolution and fast mass transport, but also is ideal for the very large DNA plasmids that would have difficulty entering the pores of a porous resin structure. These columns are effective in separating various species within a short run, which presents an interesting strategy for complex samples (**click** for further reading).

Denaturing conditions obtained with high-pH eluent eliminate Watson-Crick hydrogen bonding and allow resolution of problem sequences, such as self-complimentary sequences or poly-G stretches. Therefore, anion-exchange chromatography at high pH has become the preferred approach for oligonucleotide analysis.

The workflow

The DNAPac PA200 column readily separates different forms of plasmids that are likely to be generated during a production stage. UV absorbance detection and the narrow ID of the column provide sample sensitivity and easy monitoring for plasmid samples. The column's capabilities to separate linear plasmids range from 5% to 50% in a supercoiled plasmid sample.

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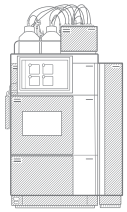
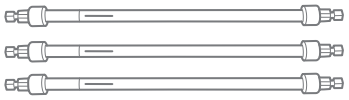
Gene therapy workflow

Plasmid DNA (pDNA) for gene therapy workflow





Instruments → **Columns** → **Vials and caps**

Workflow solutions

Description	Quantity	Cat. no
Thermo Scientific instruments		
Thermo Scientific Vanquish Flex Quaternary UHPLC system	Each	IQLAAAGABHFAPUMBHV
Thermo Scientific columns		
DNAPac PA200 oligonucleotide HPLC column, 8 µm, 2.0 × 250 mm	Each	063425
Thermo Scientific vials and caps		
Thermo Scientific™ SureSTART™ 0.3 mL Polypropylene Vial	100/pack	6ESV9-04PP
Thermo Scientific™ SureSTART™ 9 mm Screw Cap	100/pack	6PSC9ST1

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Adeno-associated virus (AAV) – viral vector introductions

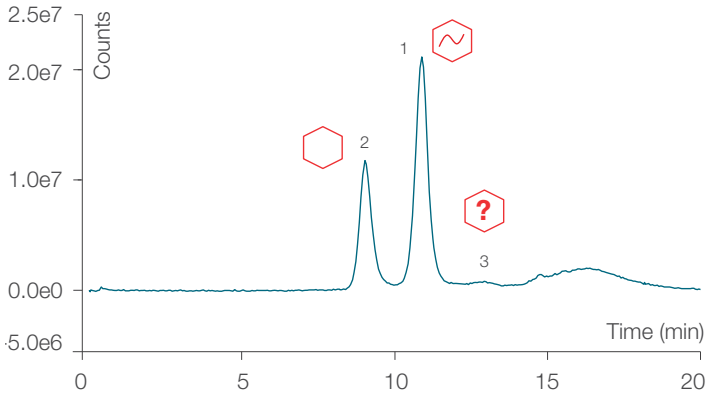
Adeno-associated viruses are the main viral vectors for gene therapy and have been successful in treating inherited retinal diseases and spinal muscular atrophy. An AAV is composed of an icosahedral protein shell with a single-stranded genome of approximately 4.7 kb. The intact AAVs act as a vehicle to protect and deliver gene therapeutics.

AAV based gene therapeutics are more complex than many traditional biotherapeutics. Aside from the full capsid containing the desired gene material, the final product could contain many different types of process- and product-related impurities. Full characterization, including sequence and PTM identification of viral proteins is required to mitigate immunogenicity and ensure the safety, quality, and efficacy of AAV products.

- The full capsid needs to be carefully characterized to ensure product efficacy
- Impurities such as host cell DNA and proteins need to be accurately characterized and controlled to ensure product quality and safety

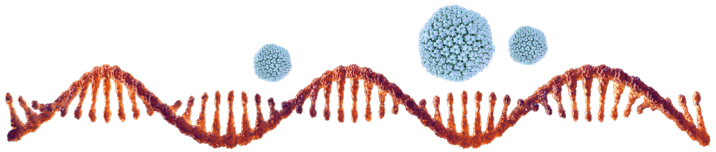
A successful viral manufacturing pipeline must deliver a consistent, pure, and high-titer product that exhibits good safety and efficacy to meet regulatory expectations.

Read this **application note** to learn more.



Column	**ProPac 3R SAX column, 3 µm**			
Format	2 × 50 mm			
Mobile phase	A: Water B: 1 M Tetramethylammonium chloride C: 200 mM BIS-TRIS propane, pH 9.0			
Flow rate	0.2 mL/min			
Injection	0.2 µL			
Temp	20 °C			
Detection	FLD (Ex: 280 nm, Em: 330 nm)			
Sample	AAV6 (2×10^{13} vg/mL)			
Gradient		**%A**	**%B**	**%C**
Time (min)	0.0	78	12	10
	1.0	78	12	10
	11.0	58	32	10
	11.1	0	90	10
	13.0	0	90	10
	13.1	78	12	10
	25.0	78	12	10

Figure 4. Separation of spiked AAV6 sample on ProPac 3R SAX column using a linear salt gradient. Peaks: 1. Full capsid, 2. Empty capsid, 3. Impurity.



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Adeno-associated virus (AAV) – viral vector introductions (continued)

The workflow solution for determination of full and empty AAV using SAX

The **Thermo Scientific™ ProPac™ 3R SAX column** enable rapid, efficient, and high-resolution separation of proteins and glycoproteins based on surface charge. Its 3 µm, non-porous particles, made of a polymer resin, offer exceptional resolving power. With improved column packing and reproducibility, thanks to consistent size distribution, this column effectively separates AAV empty and full capsids and other impurities using linear salt gradients. The unique design ensures high resolution, robust performance, and lot-to-lot reproducibility required for AAV analysis.

With this workflow, ProPac 3R SAX columns easily resolved full and empty AAV capsids with high resolution and high accuracy in under 12 minutes with excellent separation of empty and full capsids and other impurities.

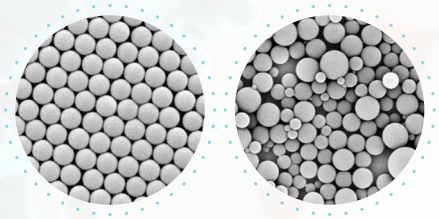

Figure 5. Scanning Electron Microscope image of 3 µm monodisperse particles (left) vs. 3 µm polydisperse particles (right).

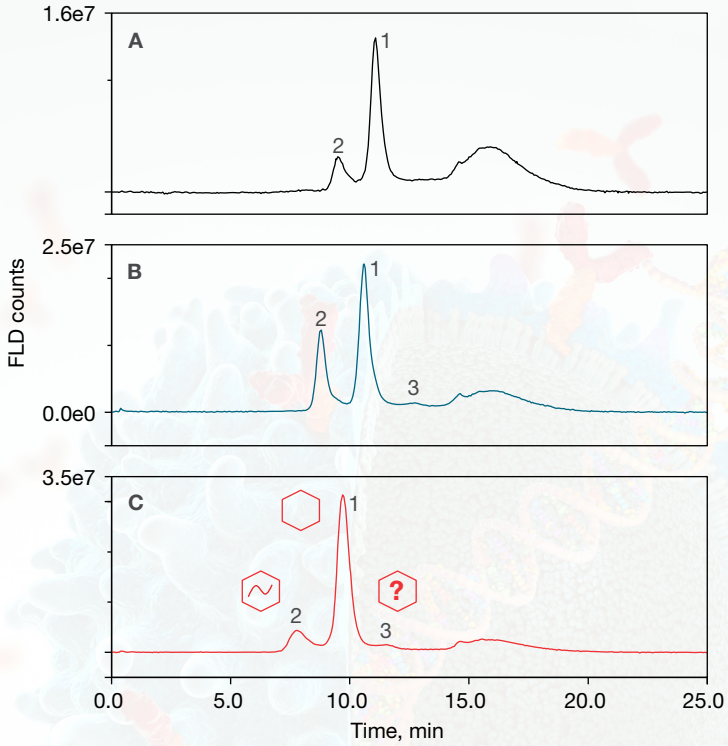

Figure 6. Linear salt gradient separation of full capsid AAV samples spiked with empty capsid to give a 1:10 Empty:Full ratio: (A): AAV1 sample, (B): AAV6 sample, and (C): AAV8 sample

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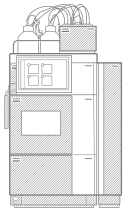
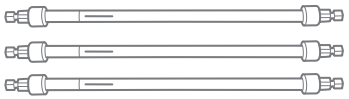
Adeno-associated virus (AAV) – viral vector introductions workflow (continued)





Instruments → **Columns** → **Vials and caps**

Workflow solution for determination of full and empty AAV using SAX

Description	Quantity	Cat. no
Thermo Scientific instruments		
Vanquish Flex Quaternary UHPLC system	Each	IQLAAAGABHFAPUMBHV
Vanquish Fluorescence Detector F with standard flow cell	Each	VF-D51-A
Thermo Scientific columns		
ProPac 3R SAX column, 3 µm, 2 × 50 mm	Each	43203-052068
Thermo Scientific vials and caps		
Thermo Scientific™ SureSTART™ 0.3 mL GOLD-Grade Clear Glass Insert	100/pack	6PME02CG
Thermo Scientific™ SureSTART™ 2 mL Vial Clear Glass (for holding insert)	100/pack	6ASV9-1P
Thermo Scientific™ SureSTART™ 9 mm Screw Cap, PTFE/silicone/PTFE septa	100/pack	6PSC9TST

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Adeno-associated virus (AAV) – viral vector introductions (continued)

The workflow solution for determination of AAV aggregates with SurePac Bio SEC MDi columns

Our groundbreaking MDi™ technology integrates our advanced monodisperse silica particle platform with biocompatible, inert hardware, ensuring superior reliability in size-exclusion chromatography. With our **Thermo Scientific™ SurePac™ Bio 550 SEC MDi™ HPLC Columns**, leverage the power of 3 µm monodisperse silica particles for robust, reproducible, and highly efficient separations and higher throughput, outperforming traditional polydisperse 5 µm media.

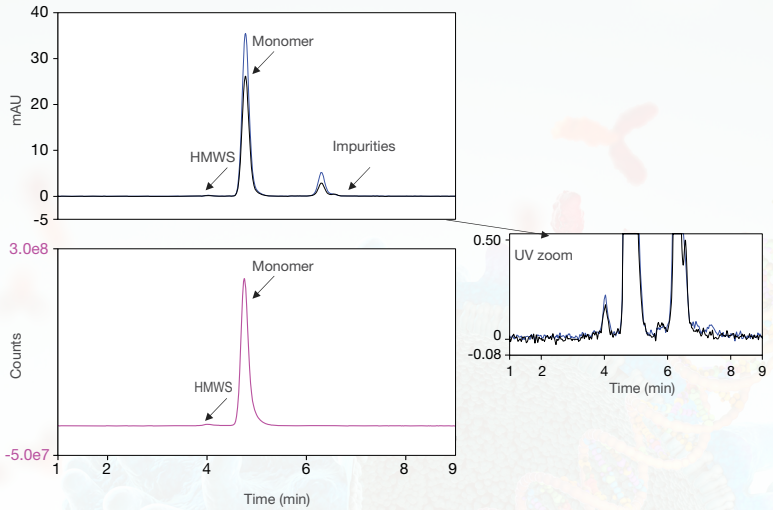
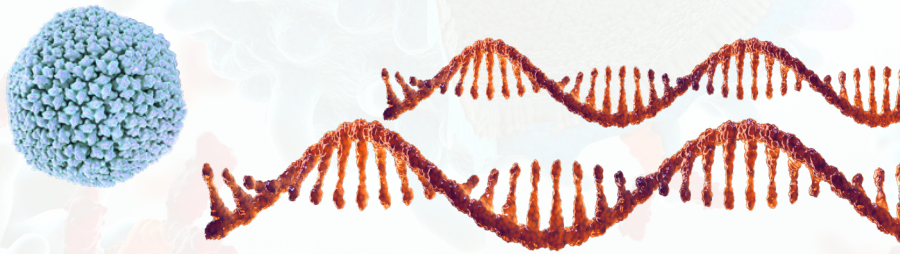


Figure 7. Separation of AAV8 monomer and high molecular weight species with UV and FLD detection.



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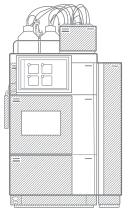

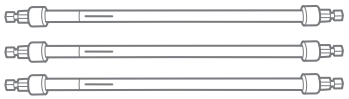

Instruments → **Columns** → **Vials and caps**

Workflow solution for determination of AAV titers using SEC

Description	Quantity	Cat. no
Thermo Scientific columns		
Thermo Scientific™ SurePac™ Bio 550 SEC MDi™ Analytical Column, 3 µm	2.1 x 150 mm	43903-152131
	4.6 x 150 mm	43903-154631
	7.8 x 150 mm	43903-157831
Thermo Scientific™ SurePac™ Bio 550 SEC MDi™ Guard Column, 3 µm	2.1 x 30 mm	43903-032131
	4.6 x 30 mm	43903-034631
	7.8 x 30 mm	43903-037831
Thermo Scientific vials and caps		
Thermo Scientific™ SureSTART™ 2 mL Polypropylene Screw Vial	100/pack	6ESV9-04PP
Thermo Scientific™ SureSTART™ 9 mm Screw Cap	100/pack	6PSC9ST1
Thermo Scientific instruments		
Vanquish Flex Quaternary UHPLC system	Each	IQLAAAGABHFAPUMBHV
Vanquish Fluorescence detector	Each	VF-D51-A
Thermo Scientific™ Vanquish™ Variable Wavelength Detector	Each	VF-D40-A

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Host cell protein and peptide mapping of AAVs and their post translational modifications

Among the quality attributes that need monitoring, characterization of the **AAV capsid protein amino acid sequences** and any associated PTM present should be performed. This full characterization also includes process-related impurities, such as residual host cell proteins (HCP) derived from the production cells to mitigate immunogenicity and ensure the safety, quality, and efficacy of AAV products.

As commonly used for recombinant protein analysis, LC-MS based peptide mapping can provide sequence coverage and PTM information and identification of HCPs to improve product development and deployment of associated manufacturing processes.

The sample preparation and columns

- **Peptide mapping** can be performed using a pepsin **Thermo Scientific™ SMART Digest™ Kit** to avoid the difficulty in digesting AAV capsid proteins with the commonly used in-solution trypsin digestion protocols, which is often slow and requires several hours
- A micro-flow LC-MS/MS method can be carried out using standard UHPLC-MS platforms by simply switching from a larger I.D. column (e.g., 2.1 mm) to a smaller I.D. column (e.g., 1 mm) to reduce the injection amount of AAV sample compared to high flow LC MS/MS, while maintaining comparable method robustness and reproducibility

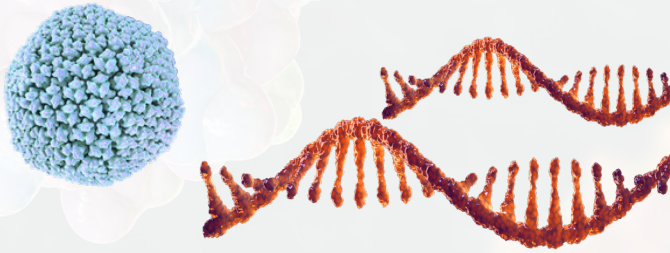
The **Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 UHPLC columns** are an excellent column choice for a broad range of peptides, offering high resolution for all critical quality attributes, without extremely long retention for more hydrophobic peptides. The column offers sub-2 µm particles providing ultra-short diffusion paths that result in extremely efficient separations.

The **Thermo Scientific™ Vanquish™ Analytical Purification LC System** include the integrated Thermo Scientific™ Vanquish™ Fraction Collector to collect the impurity peaks of interest for further characterization like digestion and peptide mapping.

The workflow

The workflow consists of:

- Robust micro-flow LC-MS/MS using the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer equipped with the Thermo Scientific Vanquish Horizon UHPLC system
- A Thermo Scientific™ Hypersil GOLD™ Peptide UHPLC column for peptide mapping of AAV viral proteins and HCP profiling of pre-purified and purified AAV samples
- Pepsin for AAV6 sample digestion for 100% AAV6 viral protein sequence coverage from a single LC-MS/MS run
- Thermo Scientific™ SOLA™ HRP solid-phase extraction cartridges or plates combined with pepsin digestion recommended to further increase sensitivity and decrease lower limits of quantitation



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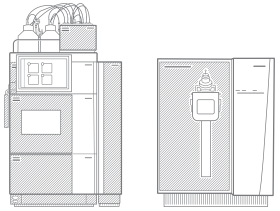
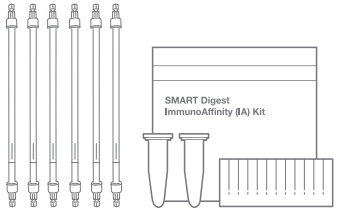
Gene therapy workflow

Host cell protein and peptide mapping of AAVs and their post translational modifications workflow (continued)





Instruments → **Columns** → **Vials and caps**

Workflow solutions

Description	Quantity	Cat. no
Thermo Scientific instruments		
Vanquish Horizon UHPLC system	Each	IQLAAAGABHFAPUMZZZ
Thermo Scientific™ Vanquish™ Neo UHPLC System	Each	VN-S10-A
Orbitrap Exploris 480 mass spectrometer	Each	BRE725533
Thermo Scientific columns and consumables		
Thermo Scientific Hypersil GOLD Peptide column	Each	26002-052130
Thermo Scientific™ PepMap™ 100 C18 Column, 3 µm, 1.0 × 150 mm	Each	164572
SMART Digest Pepsin kit	Each	60109-110
Thermo Scientific™ SOLAµ™ HRP SPE 96 Well Plate, 2 mg/1 mL	Each	60209-001
Thermo Scientific™ SOLA™ HRP SPE Cartridge, 10 mg/1mL	100/pack	60109-001
Thermo Scientific vials and caps		
SureSTART 2 mL polypropylene vial	100/pack	6ESV9-1PP
SureSTART 9 mm screw cap	100/pack	6PSC9ST1

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Introduction

Oligonucleotide therapies are short, single- or double-stranded DNA or RNA molecules used to enhance or repress the expression of target RNA, in order to treat or manage a wide range of diseases. Oligonucleotide therapeutic modalities include antisense oligonucleotides (ASO), short interfering RNA (siRNA, RNAi) and aptamers.

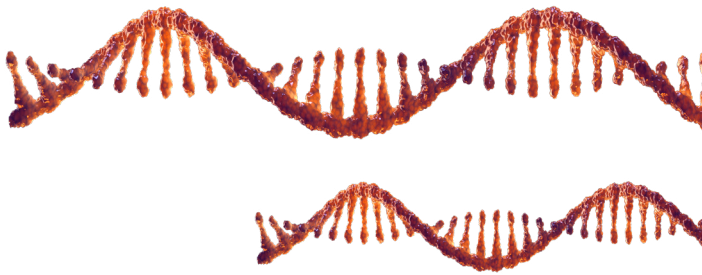
Growing interest in oligonucleotides is driven by an increased focus on personalized medicine and on the development of therapies for rare diseases. The increased demand for oligonucleotides requires a cost-effective and easy scale-up from research amounts to commercial needs.

Oligonucleotide therapeutics are difficult to produce in high yields at high purity, and difficult to formulate for efficient delivery and stability. As the pipeline for oligonucleotide-based therapeutics continues to expand, there is a growing need for analytical tools and workflow solutions that deliver robust and accurate analytical characterization to confirm identity, and to determine purity, quality, and strength to improve efficiency, and enable regulatory compliance.

Chromatographic methods are part of the typical purification schemes of therapeutic oligonucleotides. The efficacy of fractionation can be determined by comparing the sample chromatogram at the detector with the resulting elution profile achieved by re-analyzing the collected fractions. Higher performance fraction collectors like the Vanquish Fraction Collector enable fractionation with such high resolution that the resulting elution profile matches exactly the corresponding peaks of the sample chromatogram.

Read this technical note and application note to learn more.

(U)HPLC methods are also applied for purity control and characterization of synthetic oligonucleotides as described in this application note.



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Because **nucleic acids** are susceptible to *in vivo* degradation, several modifications to oligonucleotide structure are applied to improve their stability and biological persistence. These oligonucleotide modifications used for ASO, siRNA, and aptamer therapeutics complicate the methods required to characterize the identity and purity of preparations of these potential therapeutics. (U)HPLC chromatographic methods are part of the typical purification schemes of therapeutic oligonucleotides process development and manufacturing for purity and impurity characterization to satisfy regulatory requirements.

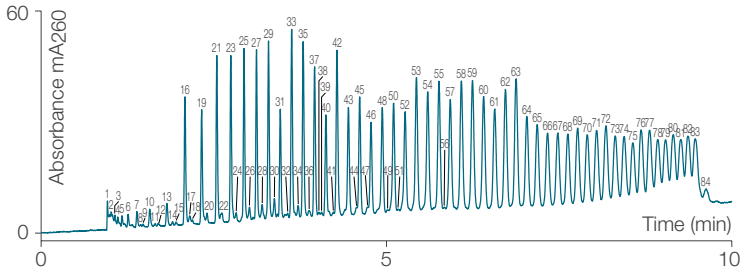

Figure 8. Separation of deoxythymidine oligodeoxynucleotides, with 84 separate components resolved in less than 10 minutes. Read this application note to learn more.

The columns

The **Thermo Scientific™ DNAPac™ PA200RS Column** is a pellicular anion exchanger resin format consisting of a porous outer shell on an impermeable resin with latex nano beads on the surface to minimize diffusion and increase resolution of ssDNA and RNA molecules. The surface exchange allows for high resolution and fast mass transport but is also ideal for the very large DNA strands that would have difficulty entering the pores of a porous resin structure. The column is also effective in separating various species within a short run due to its ion exchange capability, which presents an interesting strategy for complex samples. The column is packed in bioinert PEEK-lined stainless steel (SST) bodies: the inner sleeve of PEEK eliminates unwanted oligonucleotide interactions with SST, while the SST shell provides the necessary support for higher pressures generated by the smaller, higher-efficiency resin beads.

Denaturing conditions obtained with high-pH eluent eliminate Watson-Crick hydrogen bonding and allow resolution of problem sequences such as self-complimentary sequences or poly-G stretches. Therefore, anion-exchange chromatography at high pH has become the preferred approach for oligonucleotide analysis.

Further analysis by coupling to MS and HRAM-MS instruments supports and extends oligonucleotide characterization.

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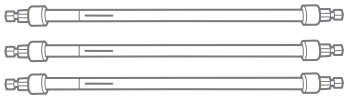
High resolution separation of oligonucleotides by ion exchange workflow (continued)



Instruments → **Columns** → **Vials and caps**

Workflow solutions

Description	Quantity	Cat. no
Thermo Scientific instruments		
Vanquish Horizon UHPLC system	Each	IQLAAAGABHFAPUMZZZ
Vanquish Diode Array Detector (DAD) FG	Each	VF-D11-A
Thermo Scientific columns		
DNAPac PA200 RS column, 4 µm, 4.6 × 150 mm	Each	082509
SMART Digest Pepsin kit	Each	60109-110
Thermo Scientific vials and caps		
SureSTART 2 mL polypropylene vial	100/pack	6ESV9-1PP
SureSTART 9 mm screw cap	100/pack	6PSC9ST1

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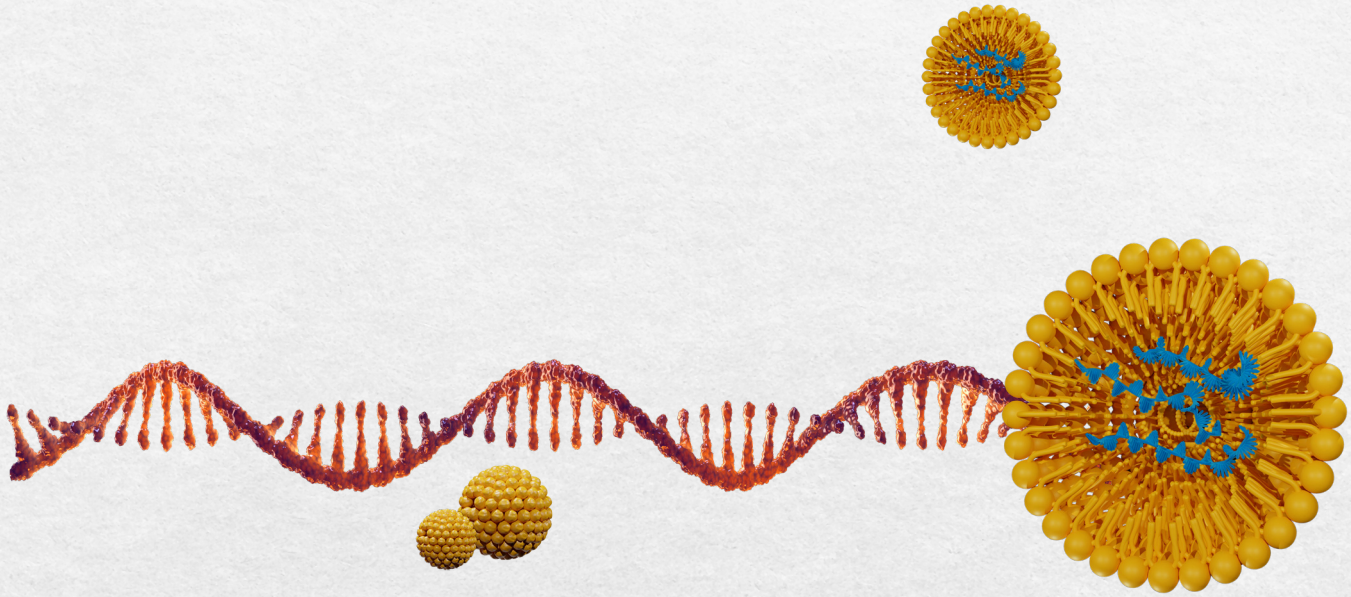
Introduction

Large **RNA**, including **mRNA**, has emerged as an important new class of therapeutics that enable the body to produce proteins needed to prevent, treat or cure disease. This was recently demonstrated with the vaccines based on mRNA sequence encoding for the modified version of the SARS-CoV-2**.** Unlike traditional biologics, mRNAs are large and delicate molecules that are produced using in vitro transcription (IVT), which need to be protected by lipid nanoparticles (LNPs) before they reach target cells.

Analytical characterization of mRNA therapeutics presents unique challenges that require new technologies and solutions.



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During the **enzymatic manufacturing process** of **mRNA therapeutics**, incomplete mRNA products are generated in conjunction with other potential impurities such as double-stranded RNA (dsRNA). Also, during manufacturing and storage, RNA and RNA therapeutics can be degraded by exposure to heat, hydrolysis, oxidation, light, and ribonucleases. It's important to assess batch-to-batch consistency of the manufacturing, process repeatability, and the quality of mRNA produced.

RNase mapping methods can be used for RNA sequence mapping to rapidly identify, characterize, and sequence map large mRNA therapeutics with high sequence coverage to provide important information for identity testing, sequence validation, and impurity analysis as is shown in the presented workflow.

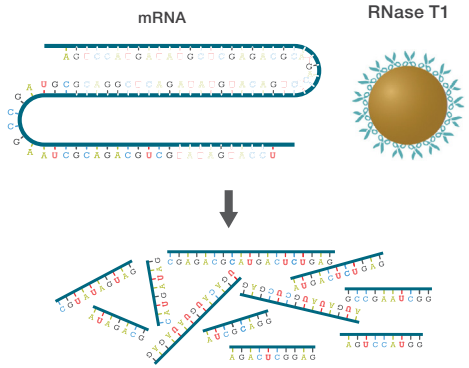

Figure 9. RNase digestions using RNase T1 immobilized on magnetic particles. Read this **document** to learn more.

The column and sample prep

Thermo Scientific™ DNAPac™ RP HPLC Column is a reversed-phase column designed for analysis of oligonucleotides and double-stranded (ds) DNA/RNA fragments using liquid chromatography-UV detection (LC/UV) or liquid chromatography-mass spectrometry (LC-MS):

- DNAPac RP resin is a unique product with a super macroporous structure consisting of both small and large pores, which provides high resolution for both short oligos and long nucleic acids. This unique column chemistry provides excellent performance under a broad range of pH (0–14), temperature (up to 100 °C), and mobile phase compositions for the separation of large double-stranded nucleic acids up to 10k base pairs.
- DNAPac is especially designed for ion-pair reversed-phase (IP-RP) separations, with a polymeric backbone bead that provides sharper peaks and less band broadening often seen with silica columns and can withstand extensive cleaning.
- DNAPac is also an excellent column for high throughput analysis on MS as depicted below.

Thermo Scientific™ SMART Digest™ RNase Kit provides a significant advance in sample preparation for oligonucleotide mapping, and provide fast and simple digestion with high reproducibility, sensitivity, and data quality in a format that's compatible with automation. With these kits, you can perform single nuclease digestion and achieve full characterization of mRNA without nuclease contamination of the analytical column.

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High resolution MS analysis mRNA sequence confirmation (continued)

The workflow

The **complete digestion of large RNA**, including mRNA therapeutics using RNases such as RNase T1/A, results in the production of a large number of small oligoribonucleotides, which map to many different locations throughout the RNA sequence and therefore do not generate unique sequences for sequence mapping. It is therefore beneficial to perform a partial RNase digestion using RNase T1 immobilized on magnetic particles, with separation achieved on a longer DNAPac RP column to assure the necessary peak capacity for such a mapping sequence.

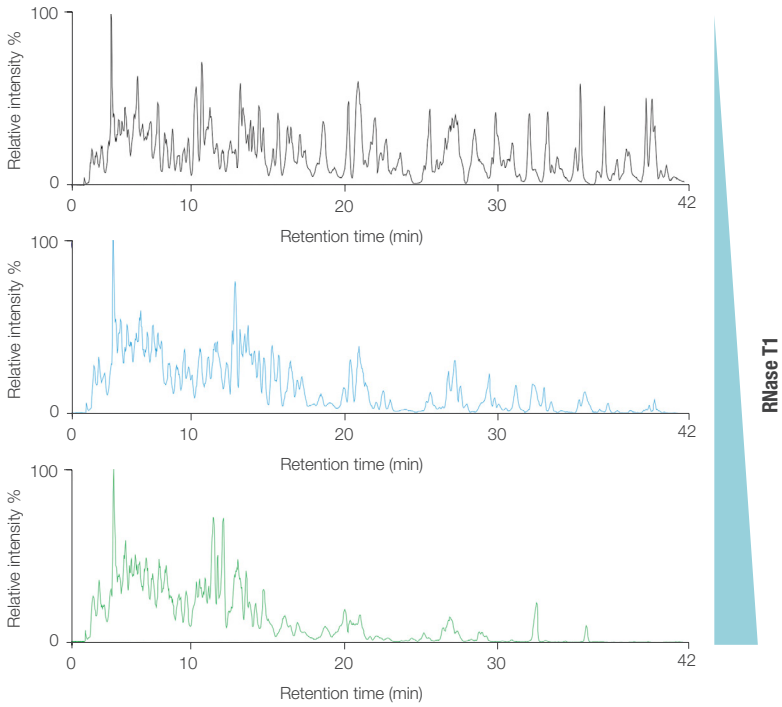

Figure 10. Optimization chromatograms of partial RNase T1 digests of mRNA. Read this publication: Vanhinsbergh at al. Anal. Chem. 2022, 94, 7339–7349

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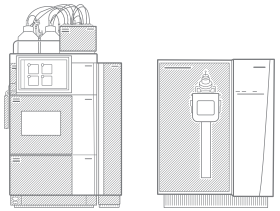
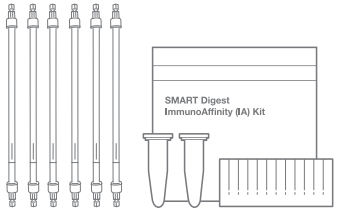
High resolution MS analysis mRNA sequence confirmation workflow (continued)





Instruments → **Columns and consumables** → **Vials and caps**

Workflow solutions

Description	Quantity	Cat. no
Thermo Scientific instruments		
Vanquish Flex Quaternary system	Each	IQLAAAGABHFAPUMBHV
Thermo Scientific™ Viper™ MS Connection Kit for Vanquish LC system	Each	6720.0405
Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer	Each	BRE725535
Thermo Scientific columns and consumables		
Thermo Scientific DNAPac RP HPLC column, 4 µm, 2.1 × 250 mm	Each	303324
Thermo Scientific™ DNAPac™ RP Guard Cartridge	Each	088925
Thermo Scientific™ SMART Digest™ RNase T1 Mag Bulk Kit	Each	60120-101
Thermo Scientific™ SMART Digest™ RNase T1 Mag Bulk Kit	Each	60120-102
Thermo Scientific vials and caps		
SureSTART 0.3 mL polypropylene vial	100/pack	6ESV9-04PP
SureSTART 9 mm screw cap	100/pack	6PSC9ST1

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PCR/IVT (monitoring IVT reaction – enzymatic)

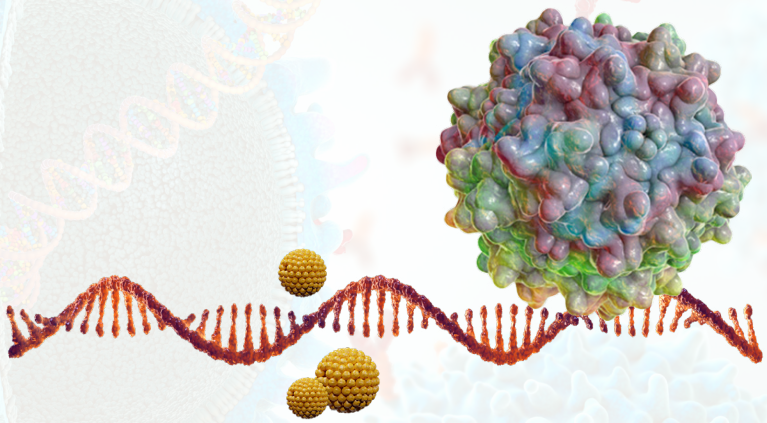
***In vitro* transcribed (IVT) mRNA** can be applied for protein replacement treatment or immunization after entering the cytoplasm. This process would not cause irreversible genome changes and induce genetic risks like DNA-based therapeutics.

During transcription, a “copy” of the expressed gene is made that carries the message encoded in the DNA. This transcript is a strand of RNA, mRNA, that once processed and exported to the ribosome, is then translated into proteins that keep cellular processes functioning. The mature mRNA is composed of five regions, each with specific biological significance: the 5’ cap, the 5’ and 3’ untranslated regions, the reading frame, and the poly(A) tail.

For *in vitro* produced mRNA, characterization of poly(A) tail length is a crucial part of transcript design. Determination of tail length assists in determination of translation efficiency, which has a direct impact on the efficacy of the therapeutic. Analytical measurements of the poly(A) tail have been mostly RNA-Seq based, relying on reverse transcription followed by amplification. These experiments can be costly due to instrumentation requirements, sample workup, and analysis.

The column and sample prep

Sample preparation digestion of IVT mRNA with **RNase T1 kit** and analysis of oligonucleotide fragments performed with a **Thermo Scientific DNAPac RP column** using liquid chromatography-UV detection (LC/UV) or LC-MS is as described in the previous section, with related application note.



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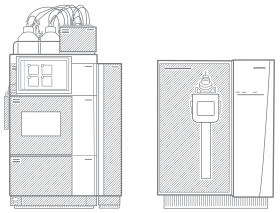
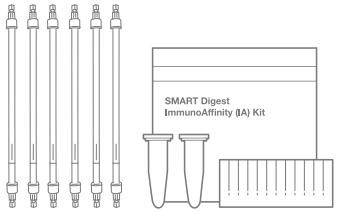
PCR/IVT (monitoring IVT reaction – enzymatic) workflow (continued)





Instruments → **Columns and consumables** → **Vials and caps**

Workflow solutions

Description	Quantity	Cat. no
Thermo Scientific instruments		
Vanquish Horizon UHPLC system	Each	IQLAAAGABHFAPUMZZZ
Viper MS connection kit for Vanquish LC systems	Each	6720.0405
Orbitrap Exploris 240 mass spectrometer	Each	BRE725535
Thermo Scientific columns and consumables		
DNAPac RP column, 4 µm, 2.1 × 100 mm	Each	088923
SMART Digest RNase T1 mag bulk kit	Each	60120-101
SMART Digest RNase T1 mag bulk kit	Each	60120-102
Thermo Scientific vials and caps		
SureSTART 0.3 mL polypropylene vial	100/pack	6ESV9-04PP
SureSTART 9 mm screw cap	100/pack	6PSC9ST1

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mRNA purity determination

After the synthesis, the **mRNA** must be purified from the remaining reaction by products. Among the most common impurities are nucleotides, enzymes, DNA templates and fragments, abortive transcript fragments, double stranded RNA (dsRNA), and primers. Read this **application note**.

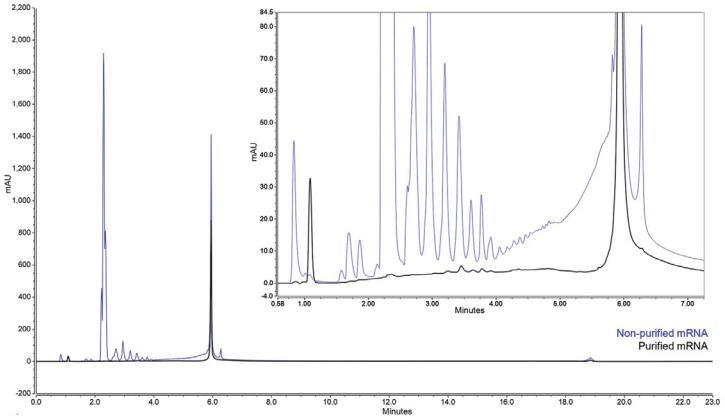


Figure 11. Chromatograms of purified versus non-purified mRNA with Thermo Scientific™ DNAPac™ PA200 RS column, 4 µm, 4.6 × 150 mm.

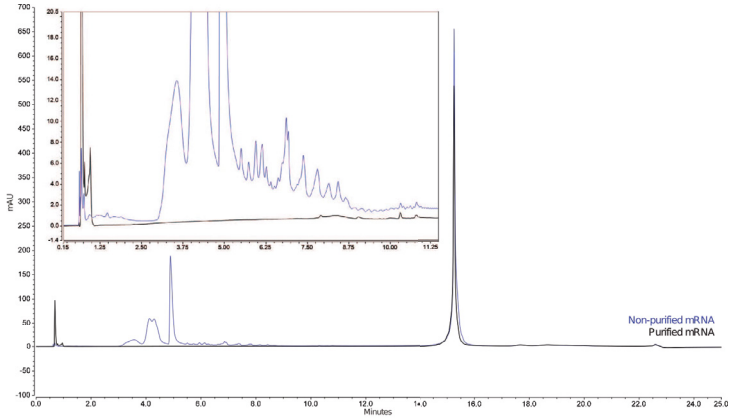


Figure 12. Chromatograms of purified versus non-purified mRNA with Thermo Scientific™ DNAPac™ RP column, 4 µm, 2.1 × 100 mm.

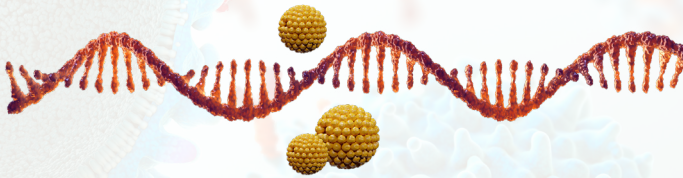
The column

The Thermo Scientific DNAPac RP HPLC columns deliver superior reversed-phase separation of oligonucleotides. The unique chemistry is designed for analysis of oligonucleotides and double-stranded (ds) DNA/RNA fragments using LC-UV or LC-MS. The column chemistry provides excellent performance under a broad range of pH, temperature, and mobile phase compositions. In addition, the wide pore size of the resin provides excellent separation of large double-stranded nucleic acids up to 10k base pairs.

The Thermo Scientific DNAPac PA200 oligonucleotide columns are anion exchange columns for high-resolution analysis and purification of synthetic oligonucleotides.

The workflow

The **Thermo Scientific™ Vanquish™ Duo UHPLC System** combined with the method scouting kit offers a valuable solution for determining the most promising chromatographic conditions in a time-effective manner. With the Vanquish Duo system, two independent chromatographic chemistries, anion-exchange and ion-pairing reversed-phase, can be scouted on the same system at the same time.



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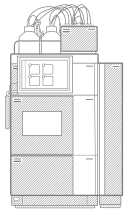

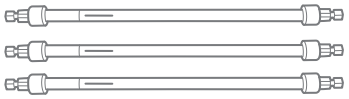

Instruments → **Columns**

Workflow solutions

Description	Quantity	Cat. no
Thermo Scientific instruments		
Thermo Scientific Vanquish Duo UHPLC system	Each	VQDUO-DUALLC
Thermo Scientific™ Viper™ Fingertight Fitting Systems Kit for Vanquish LC Systems	Each	6036.0100
Thermo Scientific columns		
Thermo Scientific DNAPac RP column, 4 µm, 2.1 x 100 mm	Each	088923
Thermo Scientific DNAPac PA200 RS column, 4 µm, 4.6 x 150 mm	Each	082509

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Characterization of lipid nanoparticle mRNA delivery vector

Irrespective of their therapeutic mechanism of action, the large size of some therapeutic RNAs, such as mRNAs, their anionic charge, and their susceptibility to RNases present in both the bloodstream and tissues, make it difficult for therapeutic RNA to enter cells efficiently and function on its own. Lipid nanoparticles (LNP) are nonviral effective delivery vehicles for the in vivo delivery of nucleic acid and vaccines are increasingly used in the field of gene therapy, protein replacement therapy, and mRNA-based vaccines developed against both cancer and infectious diseases.

The delivery of the mRNA molecule requires cellular uptake of the LNP delivery system followed by endosomal escape of the mRNA into the cytosol of the targeted cell to start the translation process.

The composition of the LNP is an important aspect for function and so must be characterized in the formulation. As such, the identification, ratio, and purity of the lipids in the formulation are regarded as critical quality attributes for safety and efficacy.

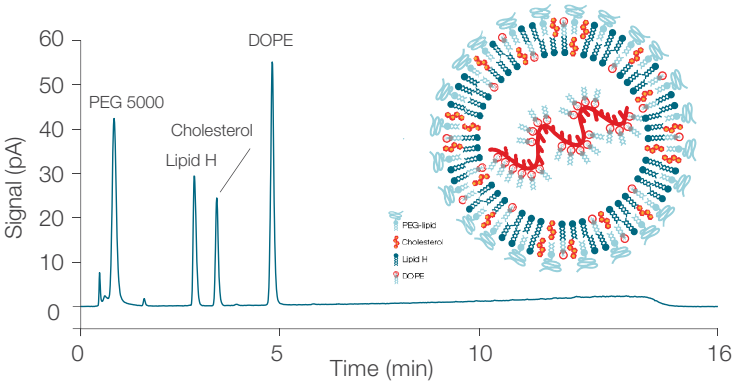

Figure 13. Separation of LNP formulation containing four different lipids - Baseline separation and detection of each lipid is obtained within 5 minutes. Read this application note to learn more.

The columns

Thermo Scientific™ Accucore™ C30 Columns are ideal for fast, high-resolution separations of hydrophobic, long-chain compounds. The Accucore columns are packed with solid core silica particles that give high separation power without high backpressures; this is important when using the more viscous eluents such as isopropanol. The C30 stationary phase offers unique shape selectivity for structurally related isomers and is compatible with highly aqueous mobile phases.

The workflow

The individual lipid components of LNPs are different in their composition and need to be separated in the chroma-tography. A cationic lipid (a) is required to interact with the phosphate backbone of the oligonucleotide to be encap-sulated. An example of the structure of 4-hydroxybutyl(azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate (DHA) is listed below, which is used in LNP formulations. A phospholipid (b), such as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), is a naturally occurring phosphatidylcholine found in cell membranes.

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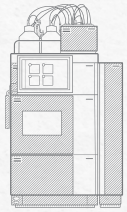
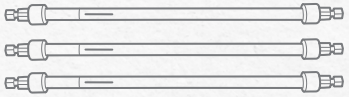
Characterization of lipid nanoparticle mRNA delivery vector workflow (continued)





Instruments → **Columns** → **Vials and caps**

Workflow solutions

Description	Quantity	Cat. no
Thermo Scientific instruments		
Thermo Scientific Vanquish Flex Binary UHPLC system	Each	IQLAAAGABHFAPUMBJC
Thermo Scientific™ Vanquish™ Charged Aerosol Detector H	Each	VH-D20-A
Thermo Scientific columns		
Accucore C30 HPLC column	Each	27826-152130
Accucore C30 HPLC guard column	Each	27826-012105
Thermo Scientific™ Uniguard™ Direct-connection Guard Cartridge Holder	Each	852-00
Thermo Scientific vials and caps		
Thermo Scientific™ SureSTART™ 1.7 mL Snap Vial	100/pack	6PRV11-S1V
Thermo Scientific™ SureSTART™ 11 mm Snap Cap	100/pack	6PRC11ST1

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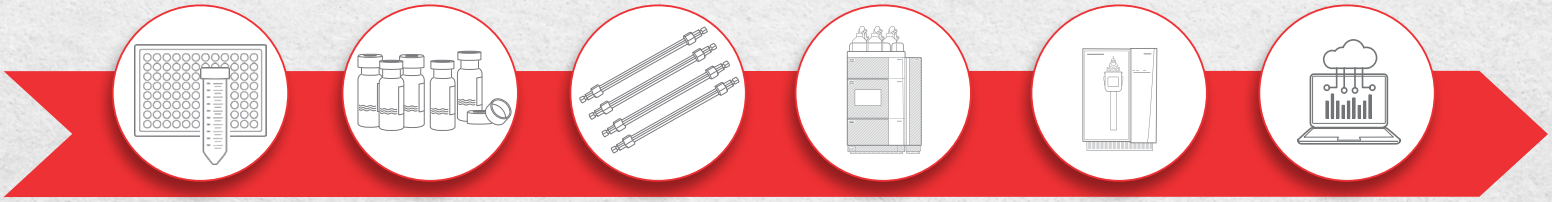
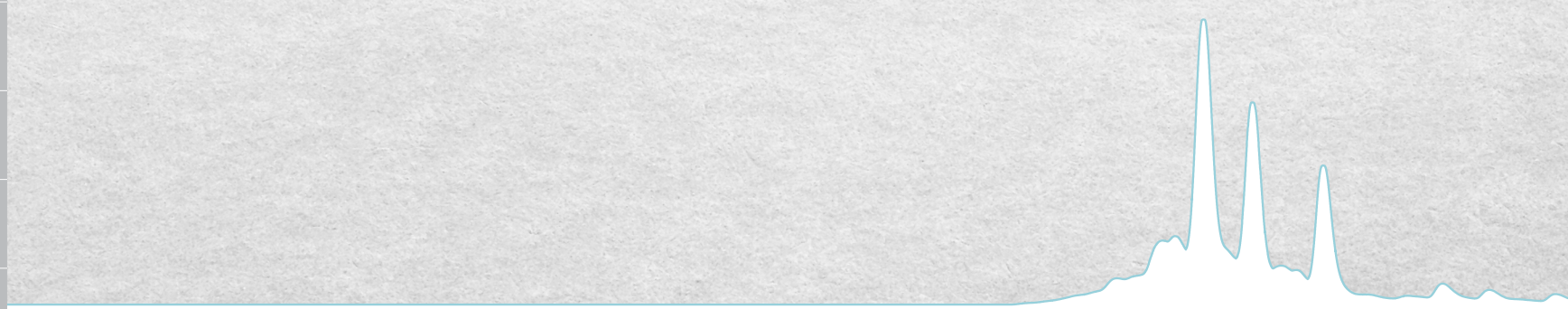


Sample preparation | Vials and caps | LC columns | LC chromatography | Mass spectrometry | Data management



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