Let the future start today
Development of better LNP-based genetic medicines
Lipid nanoparticles (LNPs) are effective non-viral vectors, which are widely used delivery vehicles for genetic medicines such as messenger RNA (mRNA). As a result, it is important to understand and limit LNP-related impurities to help ensure genetic product integrity and mitigate risks to patient safety and product efficacy. To keep up with global demand, there is also a need to shorten development timelines for gene-based therapeutics and vaccines.

Discover how you can accelerate the time to patient for new vaccines and therapies with innovative, streamlined technology that is quicker and more intuitive.
What the experts say

Adam Crowe,
Manager Analytical Development at Precision NanoSystems Inc.

“LNPs pose unique analytical challenges, in part due to the complexity of their lipid excipients. Consequently, the detailed structure elucidation capabilities afforded by electron activated dissociation [EAD] in the SCIEX ZenoTOF 7600 system provide the analytical chemist unparalleled capacity to identify problematic oxidative impurities inside ionizable lipids, and thereby de-risk the LNP therapeutic development process and expedite paths to the clinic.”

Henry Kang,
Sr Research Associate at Precision NanoSystems Inc.

“Maintaining mRNA integrity is essential for the manufacture of high-quality LNP vaccines. However, predicting LNP potency from legacy, low-resolution techniques remains challenging. In contrast, the SCIEX PA 800 Plus system equipped with the RNA 9000 Purity & Integrity kit enables robust, high-resolution integrity profiling of mRNA, even for larger constructs, removing a long-standing obstacle in mRNA LNP quality control.”
Small but mighty: critical quality attributes (CQAs) of LNPs

Genetic material
The genetic cargo—such as messenger RNA (mRNA), self-amplifying RNA (saRNA), CRISPR/Cas9-related guide RNA (gRNA), small interfering RNA (siRNA), anti-sense oligonucleotides (ASOs), aptamers and transfer RNA (tRNA)—must be handled with care to help ensure its integrity.

mRNA 3’ poly-A tail
The tail of multiple adenosines at the 3’ end of mature mRNA is another CQA, protecting it from degradation and facilitating translation.

mRNA 5’ cap
The 5’ cap is a CQA that prevents degradation of the mRNA and promotes its translation.

Structure
Nucleic acids can form secondary structures due to complementary pairing of bases. These structures could affect the encapsulation or the stability of the LNPs.

Lipids
Keeping the fragile cargo safe and facilitating cellular update are important tasks of lipids used in lipid nanoparticles (LNPs).

Linear DNA
Some genetic medicines are based on linear DNA templates. Their quality is key for high product yields.

Proteome
Proteins work in complex networks. Editing a specific gene and its expression can have various effects on the entire proteome, which need to be understood.
The starting point: linear double stranded DNA (dsDNA)

RNA as a medicine started a paradigm shift with unprecedented potential. Since linear dsDNA serves as a template for in vitro transcription (IVT) of mRNA and saRNA, its size confirmation with excellent resolution and sensitivity is needed to streamline differentiation from other variants. For subsequent transfer of assays into quality control, high precision is key.

- Determine the size of your linear dsDNA with the dsDNA 1000 kit and the PA 800 Plus system
- Rely on baseline resolution of different topological variants when working with plasmid DNA (pDNA)
- Achieve the highest sensitivity for early-stage development samples with laser-induced fluorescence (LIF) detection
- Streamline the transfer of assays to quality control with the highest precision and robustness with the PA 800 Plus system

Differentiate different topological plasmid variants

Rely on a high-resolution method with optimal reproducibility
Understanding mRNA purity and integrity

Larger modalities based on nucleic acids, such as mRNA-based vaccines, can lose their efficacy when being truncated. In addition, process-related nucleic acid impurities pose a safety concern. The estimation of genome or construct size and assessment of impurities in an efficient and reproducible manner is key to increase speed to market.

Confirm genome or construct sizes up to 9,000 nucleotides (nt) and beyond with the RNA 9000 Purity & Integrity kit, compatible with the BioPhase 8800 system and the PA 800 Plus system.

Understand your product quality by profiling relevant nucleic acid impurity sizes simultaneously.

Reduce method development timelines and speed up analyses of samples by running 8 capillaries in parallel with the BioPhase 8800 system.

Confirm genome or construct size and monitor impurity profiles

Speed up your projects with 8 capillaries and the highest reproducibility
**Assessment of CRISPR/Cas9 RNA purity**

Gene editing through CRISPR/Cas9 requires the transfection of target cells with Cas9 mRNA and target-specific gRNA. For therapeutic purposes, the naturally occurring two gRNA molecules are commonly engineered into a single gRNA (sgRNA) and co-transfected with the mRNA, coding for the Cas9 protein. Both RNA molecules differ extensively in size by several thousand nt.

- Be confident in the quality of both Cas9 mRNA and gRNA with the same capillary and the RNA 9000 Purity & Integrity kit spanning 50 nt to 9000 nt.
- Get answers faster with 8 capillaries in the BioPhase 8800 system powered by intuitive software.
- Dig deeper for gRNA with the highest resolution and using the ssDNA 100-R kit and LIF detection.

**Confirm mRNA and gRNA sizes and monitor impurity profiles**

![Graph showing RNA size standard, sgRNA and Cas9 mRNA](image)

**Confirm genome sizes and monitor impurity profiles**

![Graph showing sgRNA (100 nt) and Impurities](image)
Understanding mRNA 3’ poly-A tail distribution

Although the poly-A tail of mRNA can be enzymatically added after in vitro transcription (IVT), it is commonly encoded in the pDNA template. The poly-A tail increases protein translation and serves as a protection from nucleases. Since the length is linked to translation efficiency, it is a CQA, and size determination is an analytical question of high relevance.

- Be covered for full-length and 3’ end assessment of your mRNA product with the RNA 9000 Purity & Integrity kit spanning from 50 nt to 9,000 nt
- Achieve higher throughput by smoothly transferring existing assays from the single capillary PA 800 Plus system to the 8 capillary BioPhase 8800 system
- Dig deeper into the dispersity of the tail up to 150 nt with the highest resolution and sensitivity using the ssDNA 100-R kit and LIF detection

Assess overall dispersity of your mRNA

Dig deeper into sequence parts with single-base resolution
Determination of oligonucleotide structures

Nucleic acids are known to form secondary and/or tertiary structures, based on complementary base-pairing of nucleic acids, which can impact their correct function. While software approaches for modeling exist, the prediction of tertiary RNA structures remains a challenge. Empirical approaches using chemical and enzymatic treatment often exhibit variation in selectivity regarding nucleotides and structures.

- Gain better insights into RNA backbone flexibility based on base-paired and unconstrained residues using the GenomeLab GeXP system
- Improve the accuracy of your structural prediction for your oligonucleotides of interest with empirical data without nucleotide or structural bias
- Drive toward better understanding of how the formulation of LNPs might be affected by RNA structures

Find hotspots in RNA backbone flexibility

Analysis with selective 2'–hydroxyl acylation analyzed by primer extension (SHAPE)

Linkage to base identity

Uracil

Adenine

Increase your confidence in structural predictions

SHAPE model

Probabilistic model
Characterization of lipids and related impurities

Ionizable lipids are the key components of LNPs, complexing the negatively charged cargo and facilitating the cellular uptake. Their quality is critical for a stable and efficient product. Even very low abundance N-oxide impurities, which are difficult to fully structurally elucidate, can lead to a loss of mRNA function. Saturation of double bonds of the lipids could impact the structure of LNPs and affect the final product.

- Fully understand the structures of your ionizable lipid components using electron activated dissociation (EAD) in the ZenoTOF 7600 system
- Differentiate between oxidated species and accurately localize double bonds or saturations
- Avoid missing relevant product excipients by leveraging a linear dynamic range >5 orders of magnitude and signal-to-noise enhancement with the Zeno trap

Exactly localize oxygen incorporations

Determine locations of double bonds and saturations with confidence
Identification and quantification of mRNA 5’ capping structures

The 5’ cap of IVT mRNA has a direct impact on its stability and translation and is therefore considered a CQA. Different capping structures are in use for mRNA-based medicines, which need detailed characterization and simultaneous relative quantification to ensure product quality. Differentiating the desired cap 1 from intermediate forms with similar molecular weight [uncapped analyte, G cap and Cap0] is therefore required.

- Differentiate the structural differences of your 5’ ends with accurate mass information using the ZenoTOF 7600 system or the X500 system series
- Confidently identify 5’ caps and intermediate products with Molecule Profiler software
- Obtain relative quantification information automatically in Molecule Profiler software, or tailor quantification exactly to your needs within SCIEX OS software

Rely on accurate information using high-quality data

Understand your product quality based on relative quantification

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<th>Name</th>
<th>Neutral Mass (Da)</th>
<th>R.T. (min)</th>
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Molecular weight and sequence determination

LNPs are specifically optimized to encapsulate their genetic cargo, which can range from short oligonucleotides (such as ASOs, siRNA and aptamers) that are ~18–30 nt in size, to medium-sized tRNA and sgRNA that are ~70–100 nt in size, to large mRNA and saRNA that are several thousand nt in size. In any case, accurate information is required to help ensure the quality of the RNA product.

- Trust your results with excellent raw data quality based on exceptional negative ionization efficiency and declustering of adducts with the ZenoTOF 7600 system and the X500B QTOF system
- Confidently confirm the sequence of your oligonucleotides with high-quality MS and MS/MS data in Molecule Profiler software
- Obtain relative quantification information tailored to your specific needs

Easily assess the molecular weight of your oligonucleotides

Confidently confirm the correct sequence of your product

![Graph showing molecular weight and sequence determination](image-url)
Proteome profiling after CRISPR/Cas9 gene editing

CRISPR/Cas9 is a highly specific tool in the modern medicine toolbox with the potential of curing genetic diseases with very high success rates. However, modifying a patient’s genes can have various effects on the phenotype, since cell networks are extremely complex and interdependent. To help ensure safe medications, it is necessary to look at the broader picture and to understand off-target effects.

- Understand the effects of genetic modifications on the proteome level in an unbiased way with data independent acquisition using Zeno SWATH DIA
- Dig deeper into changes with a limited sample amount with the highest level of sensitivity using the ZenoTOF 7600 system
- Determine the effects on certain proteins using simultaneous identification and relative quantification of proteins

Confirm the successful treatment of your target on the proteome level

Understand additional effects on other proteins

Data courtesy of COBO Technologies, Denmark
Consumables and software to meet your needs

- The RNA 9000 Purity & Integrity kit covers your RNA analyses from 50 nt to beyond 9,000 nt
- An 8 capillary cartridge speeds up capillary gel electrophoresis-based analyses
- Accelerate the processing of MS and MS/MS data of your oligonucleotides with Molecule Profiler software
- Experience SCIEX OS software: a powerful yet intuitive software for MS data acquisition, visualization and quantification
Hardware to help answer your questions

ZenoTOF 7600 system
This mass spectrometer gives richer, more comprehensive data based on the innovations of EAD and the Zeno trap.

X500B QTOF system
This accurate mass system combines robustness and reliability with powerful yet intuitive software to get answers faster and easier.

BioPhase 8800 system
Run multiple samples in parallel and accelerate the development and execution of sensitive, high-throughput analytical methods with 8 capillaries and the latest intuitive software.

PA 800 Plus system
Perform consistent and compliant biotherapeutics characterization with confidence.

GenomeLab GeXP system
Take control of your data and get the flexibility of a DNA sequencer and qPCR system—all in one.
SCIEX Now support network

**SCIEX Now**

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