

# Get the skinny on LNPs with Stunner

## Introduction

Particle sizing with dynamic light scattering (DLS) is one of the key analytical techniques for lipid nanoparticles (LNPs), but many DLS instruments can only measure one sample at a time and only deliver answers on size. That slows down the development of potentially life-saving gene therapies and mRNA vaccines.

Dye-based fluorescence assays used to determine RNA concentration in RNA-LNPs are everywhere, but they're nobody's favorite tool. The detergents used to destabilize LNPs can interfere with fluorescence, the assays have a limited dynamic range, and the dyes can be quite expensive. UV/Vis absorbance has been around forever for RNA quantification in purified samples, but the cloudiness of LNP samples stop most UV/Vis spectrophotometers from getting accurate readings.

Stunner pairs up high throughput DLS with intelligent, short pathlength UV/Vis to quantify size and total RNA even on cloudy RNA-LNP samples.

Stunner is the first platform to give researchers a full read-out on RNA-LNP size, size distribution, total RNA concentration and turbidity in about a minute using only 2  $\mu$ L (Figure 1). Stunner reads both UV/Vis and DLS on up to 96 samples of LNPs in just 1 hour. When even higher throughput is needed, Stunner is automation-friendly with its micro-volume SBS format plates. For regulatory environments, Stunner can be souped-up with 21 CFR Part 11 tools and UV/Vis performance can be verified for US and European Pharmacopeia compliance.

Stunner makes RNA-LNP assessment faster and easier, making it possible to check more RNA constructs, lipid mixes or formulations. A read on Stunner before a dye-based method saves the step of looking for total RNA or can take the guesswork out of choosing a dilution factor. For those burning urgent questions, a quick check of size or turbidity can spot sample degradation that



Figure 1: Stunner: the one-of-a-kind combination of nucleic acid quantification and particle sizing.

would've sabotaged your results. Using Stunner to judge RNA or LNP particle concentration plus size gives a faster, more precise and fuller picture of your sample that frees you up for the next experiment.

This app note describes how Stunner determines the total RNA concentration, particle size, size distribution and turbidity of RNA-containing lipid nanoparticles (LNPs).

## Methods

Fluc-mRNA-LNPs and empty LNP stock solutions were generously provided by Precision NanoSystems Inc (Vancouver, BC), along with particle concentrations and RNA concentrations by RiboGreen<sup>®</sup> assay.<sup>1</sup> LNPs were assembled from GenVoy-ILM<sup>™</sup> Lipid Mix and mRNA encoding luciferase using the NanoAssemblr<sup>®</sup> Ignite System. LNPs were diluted into phosphate-buffered saline (PBS), pH 7.4, and measured using Stunner's RNA-LNP app. RNA concentration was determined based on the deconvoluted absorbance in the sample due to RNA at 260 nm and with a concentration factor of 40. All Stunner measurements were performed with 6 replicates and 2  $\mu$ L of sample. A viscosity of 1.002 and refractive index of 1.334 at 20°C for PBS was used for DLS calculations.

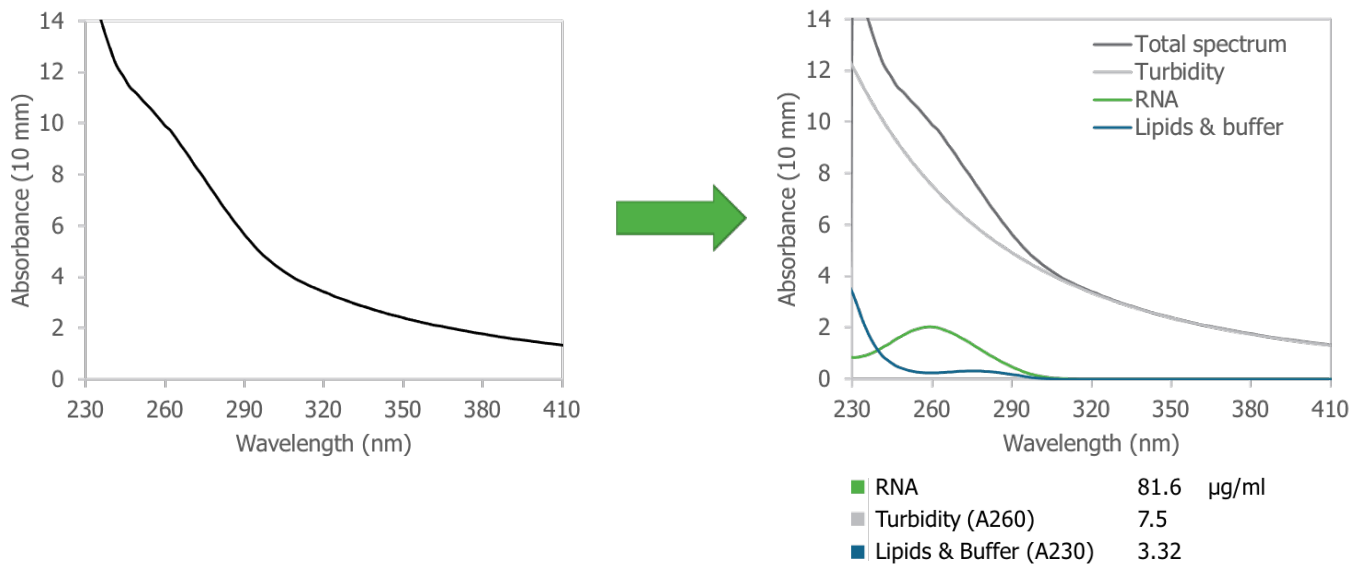


Figure 2: Stunner separates the contributions of turbidity, RNA and other components from the total UV/Vis absorbance spectrum of RNA-LNPs and uses that information to quantify total RNA concentration from 2 µL of sample.

## Results

LNPs have UV/Vis absorbance spectra dominated by light scattering across the entire ultraviolet and visible range, making it difficult or impossible for most UV/Vis spectrometers to determine RNA concentration from these challenging samples. This can be seen by eye as high levels of turbidity or cloudiness. Stunner uses Unmix algorithms to deconvolute the absorbance spectrum of RNA-LNPs and determine the individual contributions of turbidity,

RNA and other components to this overall signal (Figure 2). Once the spectrum is deconvoluted, Stunner quantifies the total RNA concentration in the sample and reports the contribution of turbidity and other components to the overall absorbance spectrum.

Dye-based RNA quantification methods for LNPs require the addition of detergents, which can impact fluorescence results, and require dilution of the sample, which can introduce error.

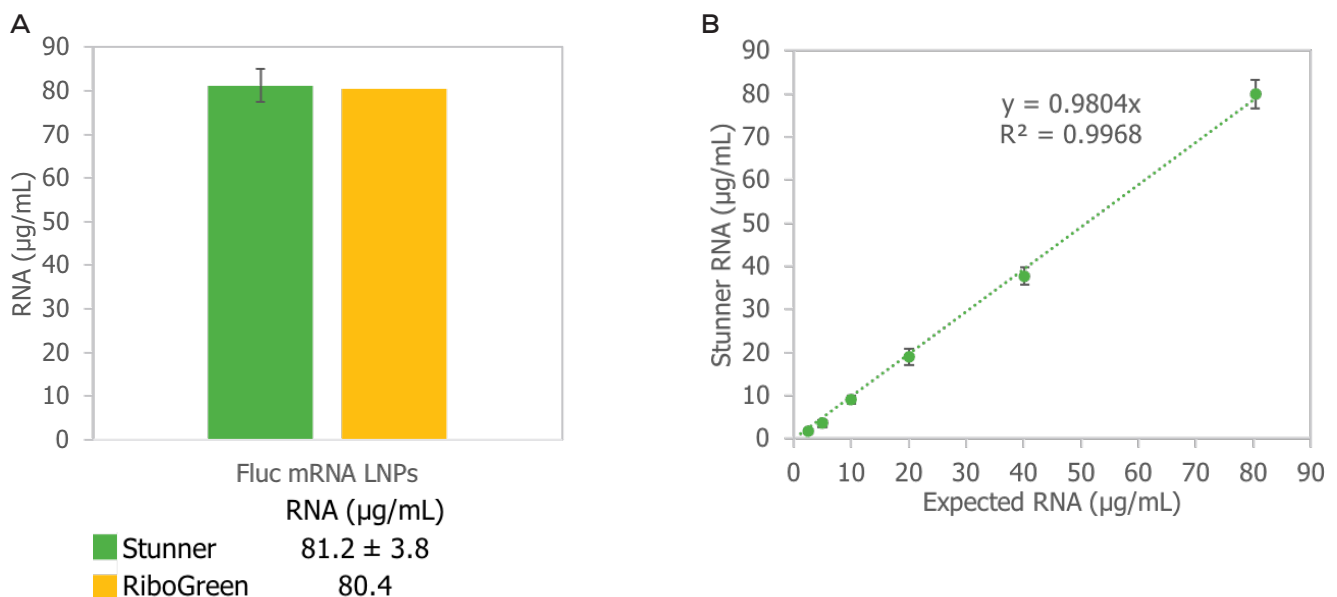


Figure 3: Stunner’s RNA concentrations agree closely with the total mRNA concentration determined by RiboGreen® (A). A 2-fold dilution series of Fluc-mRNA-LNPs showed highly linear results down to 1.2 µg/mL (B). Error bars are +/- 1 standard deviation (SD).

Stunner is a dilution-free and reagent-free quantification method, comparing favorably to a RiboGreen® assay for total RNA concentration (Figure 3A). When measuring lower concentration samples, Stunner quantifies a broad range of RNA-LNP concentrations with high precision. A 2-fold dilution series of Fluc-mRNA-LNPs showed a high degree of linearity and agreement with the expected RNA concentration with  $R^2 > 0.99$  and a slope close to 1 (Figure 3B).

Verifying particle size with DLS is a key analytical technique for lipid nanoparticles, especially for formulation and stability studies.<sup>2</sup> Other DLS instruments require significant volumes of sample or can only measure one sample at a time, making it difficult to screen large numbers of formulations or LNPs. Stunner performs DLS measurements on up to 96 samples in less than 1 hour and presents the results in an overview so average size and size distributions can be quickly compared by eye (Figure 4). Numerical results can also be exported to Excel for further analysis or saved as a PDF report, simplifying otherwise complex experiment workflows and data management.



Figure 4: Stunner's DLS determines the size and size distribution on 96 samples of LNPs in about 1 hour.

Fluc-mRNA-LNPs had an average hydrodynamic diameter of 79 nm and a CV of 1% with a polydispersity index (PDI) of 0.14 and a SD of 0.02, based on 6 replicates (Figure 5). Monodispersed LNPs samples have a  $PDI \leq 0.1$ , while highly polydispersed samples tend to have a  $PDI \geq 0.2$ . The intermediate PDI and single intensity distribution peak indicates this LNP has moderate heterogeneity in particle size. Stunner provides consistent DLS results so you can assess LNP quality with confidence.

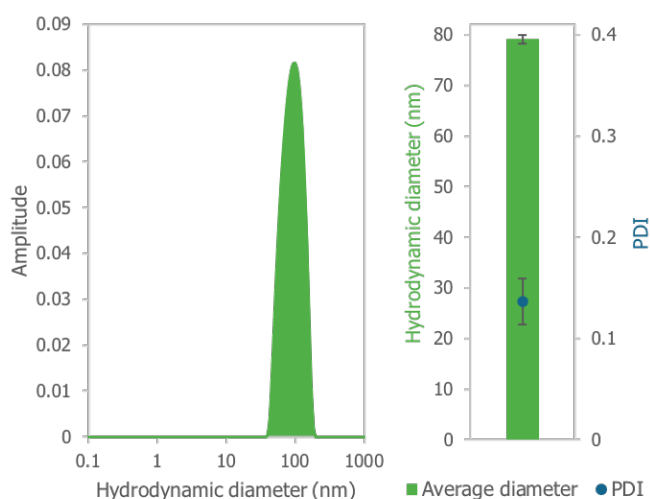


Figure 5: These Fluc-mRNA-LNPs had a hydrodynamic diameter of  $79 \text{ nm} \pm 1\%$  and a PDI of  $0.14 \pm 0.02$ .

Particle concentration is another critical attribute for the assessment of LNPs.<sup>3</sup> As part of its deconvolution of the UV/Vis absorbance spectrum of LNPs, Stunner determines the OD of the sample due to turbidity and presents it as Turbidity (A260). Turbidity of a colloidal system is determined by both the size and the number of particles in the suspension, correlating linearly to the expected particle concentration of empty and full LNPs (Figure 6). With well-defined standards, it may be possible to use Stunner's turbidity determination as method of determining particle concentration in LNP suspensions.

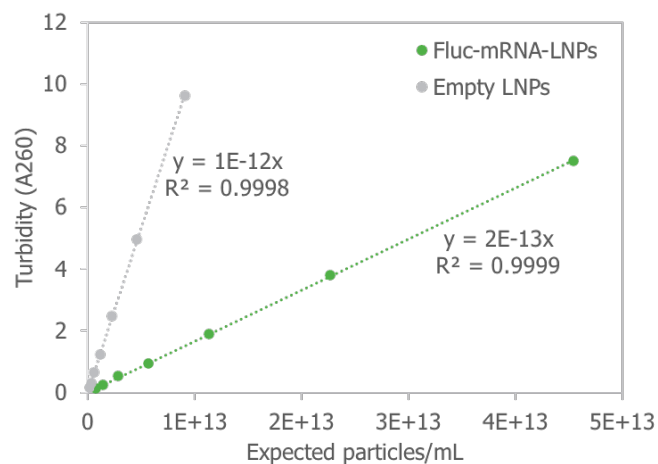


Figure 6: Turbidity correlates well with expected particle concentration for LNPs but the slope varies depending on the size and composition of the LNP, including the presence of nucleic acids.

## Conclusion

One-at-a-time DLS analysis slows down vital RNA-LNP experiments and makes it harder to know if the nanoparticles have aggregated or degraded. That slows progress on life-saving gene therapies and RNA vaccines. Stunner provides low volume, high-throughput DLS on up to 96 samples at a time and gives average size and size distribution in 1 hour. Along with highly reproducible DLS results, Stunner reduces the need for dyes, detergents and standards with reagent-free total RNA quantification. Turbidity results can give particle concentrations when combined with a standard. Regardless of whether an RNA-LNP is in research, development or manufacturing, Stunner gives you the data you need fast and helps you unravel the complexities of your samples.

## References

1. RiboGreen assay protocol, C. Walsh, et al. *Precision NanoSystems User Guide* PNI-SOP-S9-001-EXT, 2016.
2. Manufacturing considerations for the development of lipid nanoparticles using microfluidics, C. Roces, et al., *Pharmaceutics*, 2020; 12(11):1-19.
3. Use of nanoparticle concentration as a tool to understand the structural properties of colloids, L. Ribeiro, et al., *Scientific Reports*, 2018; 8(1):1-8.



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