Application note Short summary

From Microfluidic Formulation to Payload Quantification: TAMARA and CloudSpec for RNA-LNP Development

RNA-LNP development relies on both **reproducible formulation** and **reliable analytical characterization** to ensure consistent product quality. Encapsulation efficiency (EE%) is a key critical quality attribute, directly influencing dose accuracy, formulation performance, and product consistency.

The **RiboGreen assay** is commonly used to determine EE% but relies on nanoparticle disruption, introducing additional sample preparation and potential variability. **CloudSpec**, based on scatter-free absorbance spectroscopy (SFAS), provides a rapid, lysis-independent alternative for RNA payload quantification.

In this work, SM-102-based polyA-LNPs were formulated using the **TAMARA** microfluidic platform (InsideTx) and characterized using **CloudSpec** (Marama Labs). The effects of the N/P ratio and TFR on particle size, PDI, and EE% were evaluated, while CloudSpec was compared with the conventional RiboGreen assay.

TAMARA produced highly reproducible RNA-LNPs, while CloudSpec provided rapid and lysis-independent RNA payload quantification.

This work was carried out by Aida López Espinar and Kirsty Smith at Marama Labs, in collaboration with Sezen Gul from Inside Therapeutics.

Physicochemical characterization of polyA-LNPs

SM-102-based polyA-LNPs were formulated using the TAMARA microfluidic platform under three formulation conditions combining two N/P ratios (6 and 1) and two total flow rates (TFR; 5 and 10 mL/min). Particle size and polydispersity index (PDI) were characterized in triplicate by DLS.

Key findings

- → **LNPs formulated at N/P 6** exhibited highly reproducible particle size (~105 nm) and low PDI (~0.13), independent of TFR (5–10 mL/min).
- → **Reducing the N/P ratio from 6 to 1** increased both particle size (~212 nm) and PDI (~0.21), reflecting the reduced availability of ionizable lipid to drive efficient nanoparticle self-assembly.
- → **Low variability across replicate batches** confirmed the robustness and reproducibility of the TAMARA microfluidic formulation platform.

TAMARA enables robust and reproducible RNA-LNP formulation while supporting systematic formulation optimization and process development.

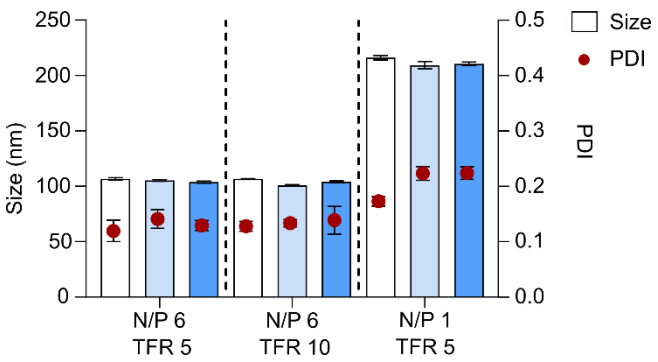
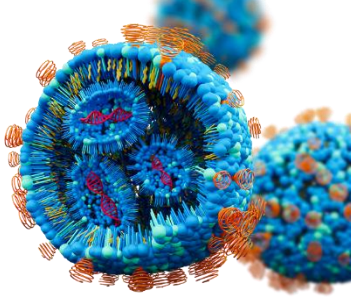


Figure 1 : Effect of N/P ratio and TFR on particle size and PDI of SM-102-based polyA-LNPs formulated using TAMARA.



Payload quantification: CloudSpec vs RiboGreen

RNA payload quantification of SM-102 polyA-LNPs was performed using the conventional RiboGreen (RG) assay and the CloudSpec-RiboGreen (CS-RG) assay. Free polyA, total polyA, and EE% were measured for each formulation condition to compare the analytical performance of both methods.

Key findings

- → **CloudSpec and the conventional RiboGreen assay showed excellent agreement** for free polyA quantification and encapsulation efficiency across all formulation conditions.
- → **CloudSpec measured total polyA concentrations closer to the expected formulation input**, while preserving the same trends between formulations and eliminating the need for nanoparticle lysis.
- → **Both methods accurately differentiated formulations with high and low encapsulation performance**, confirming the effect of decreasing the N/P ratio on RNA encapsulation.

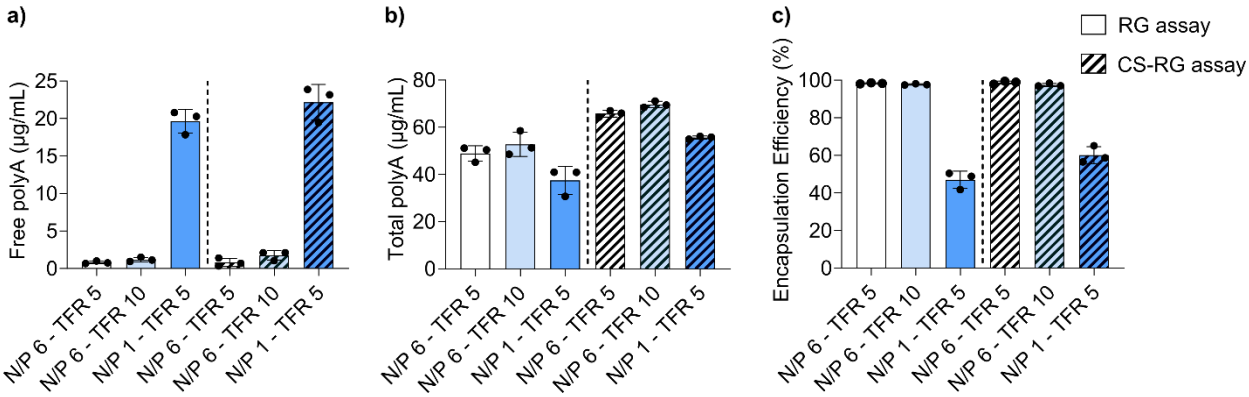


Figure 2: Comparison of RNA payload quantification using the conventional RiboGreen (RG) assay and the CloudSpec-RiboGreen (CS-RG) assay. (a) Free polyA concentration, (b) total polyA concentration, and (c) encapsulation efficiency (EE%) for SM-102-based polyA-LNPs.

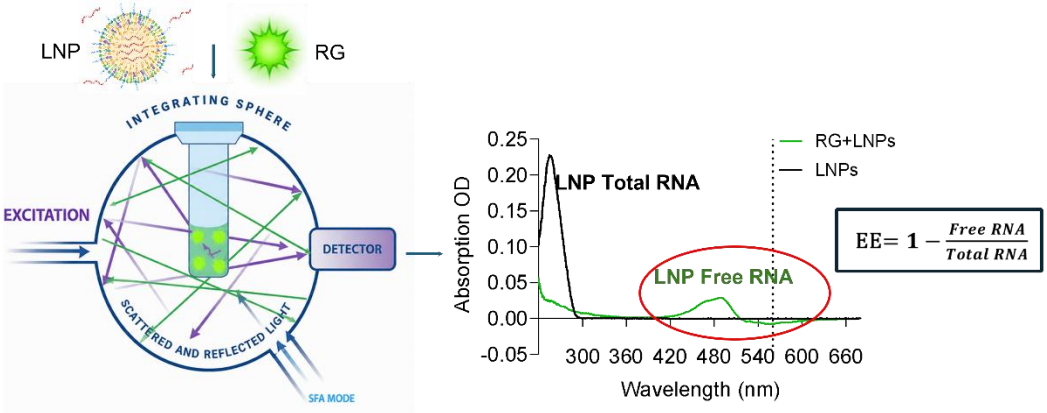


Figure 3: CloudSpec measurement principle for lysis-independent RNA payload quantification. The integrating sphere collects transmitted, scattered, and fluorescence-emitted light, enabling determination of total RNA by scatter-free absorbance spectroscopy (SFAS) and free RNA by fluorescence. A representative spectrum and the corresponding EE% calculation are shown.

CloudSpec enables rapid, lysis-independent RNA payload quantification, providing free RNA and EE% measurements consistent with RiboGreen while measuring total RNA closer to the expected formulation input.

Conclusion

This application note demonstrates the complementary value of TAMARA for **reproducible RNA-LNP formulation** and CloudSpec for **robust RNA payload quantification**.

- → **TAMARA** produced highly reproducible RNA-LNPs and enabled systematic evaluation of key formulation parameters, including the N/P ratio and TFR.
- → **CloudSpec** generated EE% measurements consistent with the conventional RiboGreen assay while providing lysis-independent total RNA quantification closer to the expected formulation input.

Together, these complementary technologies support a more efficient and reliable approach to RNA-LNP development, from formulation to payload quantification.

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