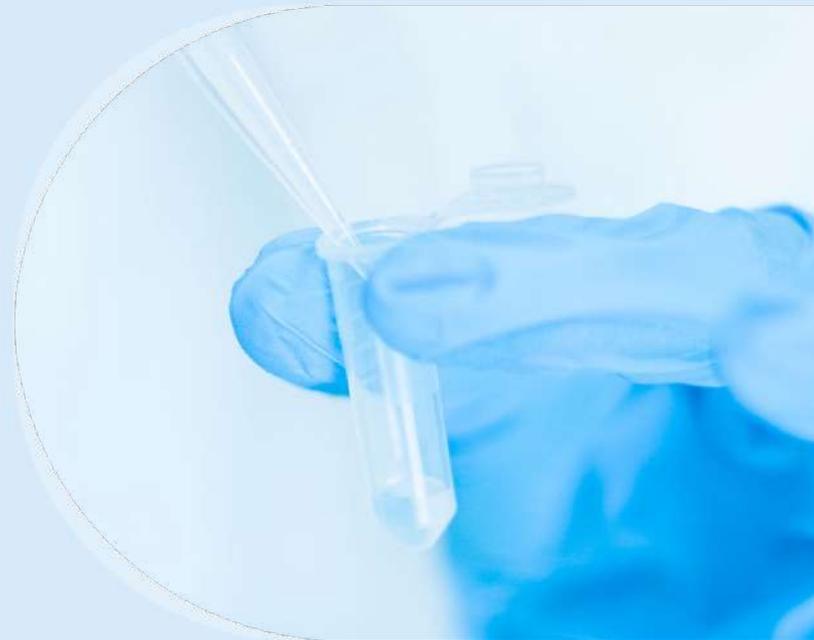


Low volume mRNA-LNP formulation screening with TAMARA

Screening of RNA-loaded lipid nanoparticles (RNA-LNPs) formulation is a critical aspect of the development of efficient mRNA-based vaccines and therapeutics. Microfluidics-based systems have emerged as the preferred solution for RNA-LNP formulation offering the advantage of minimizing very expensive reagent consumption while providing precise control over key nanoparticle characteristics.

This study provides a comprehensive evaluation of the TAMARA nanoparticle formulation system, focusing on its performance at low volumes (200, 400 and 700 μ L) to optimize RNA-LNP formulations while minimizing the use of costly mRNA. It aims at determining the extent to which the TAMARA system can **minimize reagent consumption while achieving consistent performance** by focusing on key RNA-LNP parameters such as particle size, encapsulation efficiency, encapsulation yield, transcription efficiency, cell viability and protein expression levels.



This work was carried out by the ART "ARNm" team, a R&D Inserm lab led by Prof. Chantal Pichon in Orléans, with significant contributions from Dr. Nabila Laroui and Dr. Milab Baroud, and supported by Inside Therapeutics

Impact of Formulation Parameters on Size & PDI

Polydispersity Index (PDI): Nanoparticle size distribution (0 homogeneous to 1 highly heterogeneous)

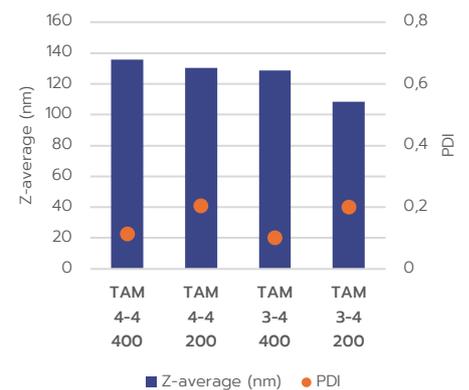
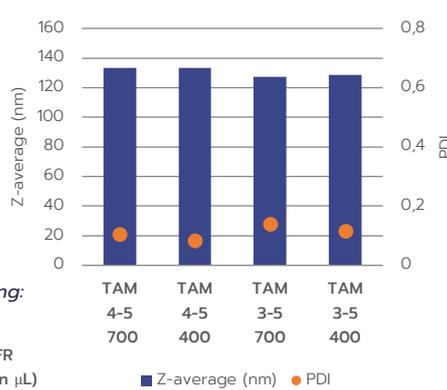
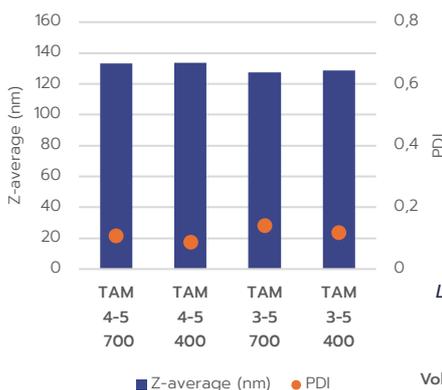
TFR and FRR impact

- Increasing the **Total Flow Rate (TFR)** reduces nanoparticle size; Flow Rate Ratio (FRR) has less impact.
- **Higher TFRs** lead to smaller particles due to efficient mixing, reducing lipid agglomeration.

Total Flow Rate (TFR): Overall flow rate
Flow Rate Ratio (FRR): The ratio between the aqueous and solvent phases.

Volume impact

- **Consistent nanoparticle sizes** between 700 μ L and 400 μ L formulations with **low PDI**.
- Challenges arise at 200 μ L, where size increases and PDI rises, indicating that **working with 400 μ L** up would be ideal.

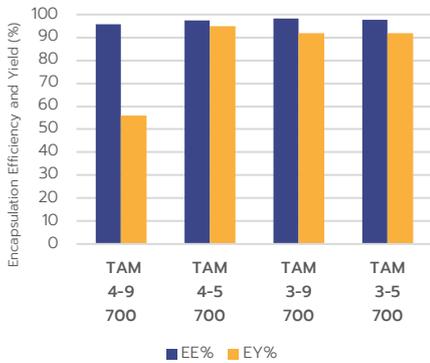


Impact on Encapsulation Efficiency (EE%) and Encapsulation Yield (EY%)

Encapsulation Efficiency (EE%): Trapped RNA/RNA at the end of the experiment
Encapsulation Yield (EY%): Trapped RNA/Input RNA. Similar to recovery

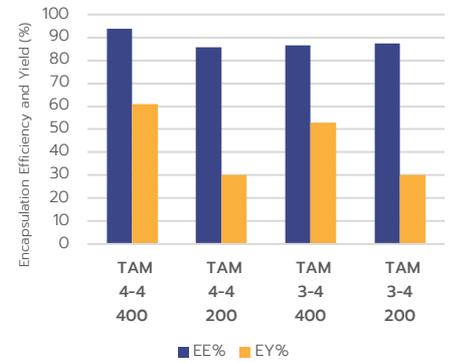
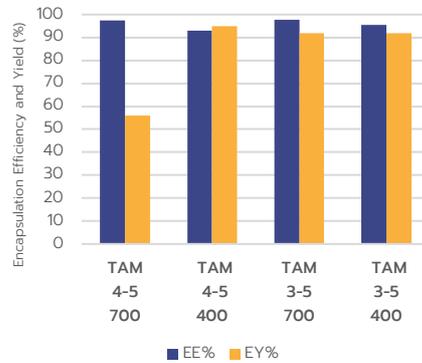
TFR and FRR impact

- **EE%:** TAMARA consistently achieved over 95% encapsulation efficiency, unaffected by formulation parameters.
- **EY%:** Encapsulation yields were also high, reaching 95%, except for one outlier (TAM 4-9 700).



Volume impact

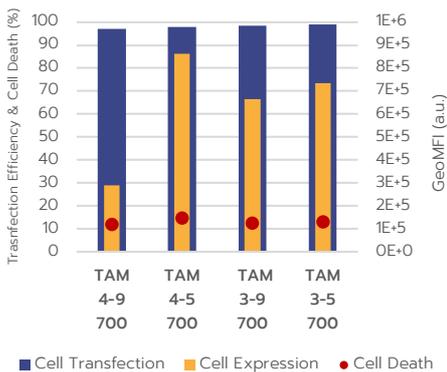
- **EE%:** Remained stable (~95%) across volumes, with a slight drop to 85% at 200 μ L.
- **EY%:** Lower volumes (200 μ L) showed reduced yields due to short run times, while larger volumes (400 μ L and 700 μ L) provided better control and higher yields.



Impact on Transfection Efficiency, Cell Viability, and Protein Expression

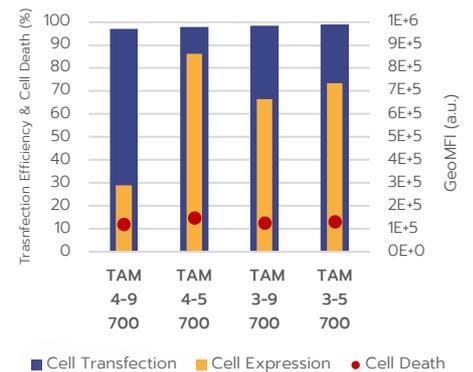
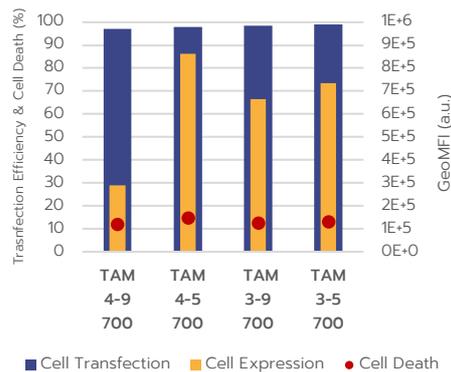
TFR and FRR impact

- **Transfection efficiency** remained consistently high (~100%) across samples.
- **Cell viability** stays high with low cytotoxicity.
- **Protein expression** generally remains high across samples.
- Formulation parameters play a major role on the final in-vitro efficiency, highlighting the **needs for screening of LNP formulation conditions/DOE**.



Volume impact

- **Transfection efficiency** stayed high (>90%) but decreased slightly with lower volumes.
- **Cytotoxicity** increased at smaller volumes, while cell viability was higher with larger volumes.
- **Protein expression** closely aligned with encapsulation yield, with 400 μ L formulations providing reliable insights into overall formulation performance.



Conclusion

- **400 μ L and 700 μ L** volumes produced consistent, high-quality results:
 - Size can be controlled using TFR; PDI remains low
 - Encapsulation efficiency remains >90%; encapsulation yield offers extremely high results
 - Cell transfection remain ~100%, protein expression highly linked to Encapsulation yield => Maximized with TAMARA.
- **200 μ L** volume with TAMARA faced challenges (lower encapsulation yield).
- **400 μ L** volume is optimal for screening, balancing material efficiency and product quality, and can be scaled up for larger studies.
- **Scalability:** following screening, mRNA-LNP Formulation with TAMARA can easily be transferred from 400 μ L to 30 mL
- **Chip usage:** All the work has been carried out with 1 single chip, cleaned and reused between runs.

contact@insidex.com
insidex.com



Read the full
Application Note

Benchmarking TAMARA against mainstream toroidal microfluidic mixer

The efficient formulation of RNA-loaded lipid nanoparticles (RNA-LNPs) plays a pivotal role in advancing mRNA-based vaccines and therapeutics. Microfluidics-based systems have become the leading technology for RNA-LNP formulation during discovery phase, thanks to their ability to minimize the consumption of expensive reagents by operating at sub milliliter scale, while maintaining precise control over key nanoparticle characteristics.

This study compares two leading microfluidic platforms for RNA-LNP formulation at < 1 mL volumes: TAMARA by Inside Therapeutics and Ignite by Precision Nanosystems/Cytiva. Their performance is evaluated using standard mRNA-LNP protocols, focusing on critical factors such as particle size, encapsulation efficiency, yield, transcription efficiency, cell viability, and protein expression. The goal is to determine which platform optimizes RNA-LNP formulation for preclinical applications.



This work was carried out together by Inside Therapeutics supported by the University of Orleans, France.

The formulation parameters for the Ignite system were previously determined and optimized through a Design of Experiments (DOE) approach, with all experiments conducted at the smallest feasible volume of 700 µL.

Influence of Formulation Parameters on Size & PDI

Polydispersity Index (PDI): Nanoparticle size distribution (0 homogeneous to 1 highly heterogeneous)

TFR and FRR impact on TAMARA

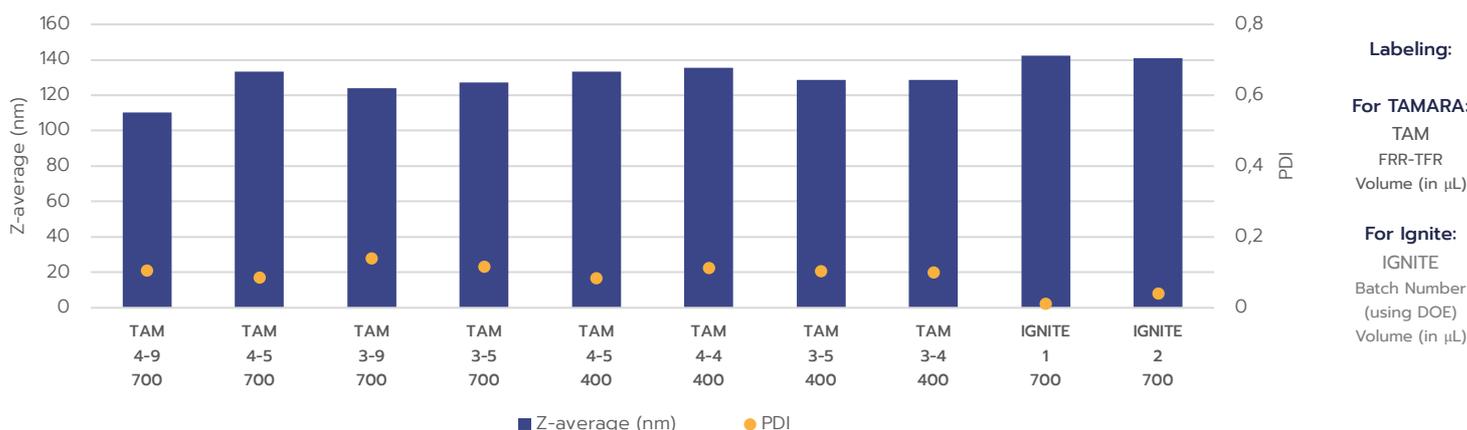
- Increasing the **Total Flow Rate (TFR)** reduces nanoparticle size; Flow Rate Ratio (FRR) has less impact.

Total Flow Rate (TFR): Overall flow rate.

Flow Rate Ratio (FRR): The ratio between the aqueous and solvent phases.

Systems comparisons

- Both systems exhibit excellent quality with size in ideal range and **PDI < 0.2 meeting industry standards.**
- Optimization of TFR & FRR parameters through DOE – such as done with Ignite - is critical to optimize formulation quality.



Labeling:

For TAMARA:
TAM
FRR-TFR
Volume (in µL)

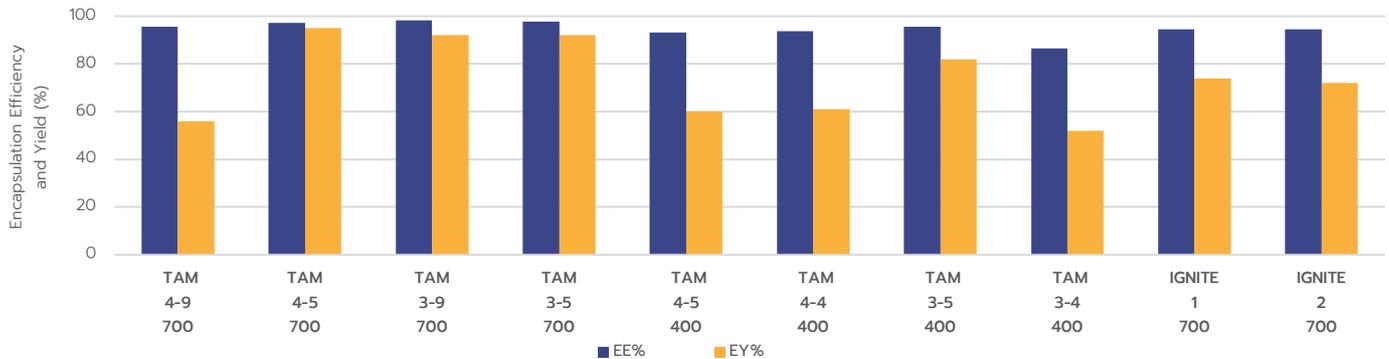
For Ignite:
IGNITE
Batch Number
(using DOE)
Volume (in µL)

Comparison of Encapsulation Efficiency (EE%) and Encapsulation Yield (EY%)

Encapsulation Efficiency (EE%): Trapped RNA/RNA at the end of the experiment
Encapsulation Yield (EY%): Trapped RNA/Input RNA. Similar to recovery

TFR and FRR impact on TAMARA

- **EE%:** TAMARA consistently achieved over 95% encapsulation efficiency, both at 700 and 400 μ L.
- **EY%:** Encapsulation yields also high, reaching 95% for 700 μ L, except for one outlier (TAM 4-9 700). Lower volumes lead to lower EY%.



Systems comparisons

- **EE%:** Both systems achieve comparable EE% levels.
- **EY%:** TAMARA demonstrates much higher level of yield than Ignite at similar volumes, maximizing RNA recovery.

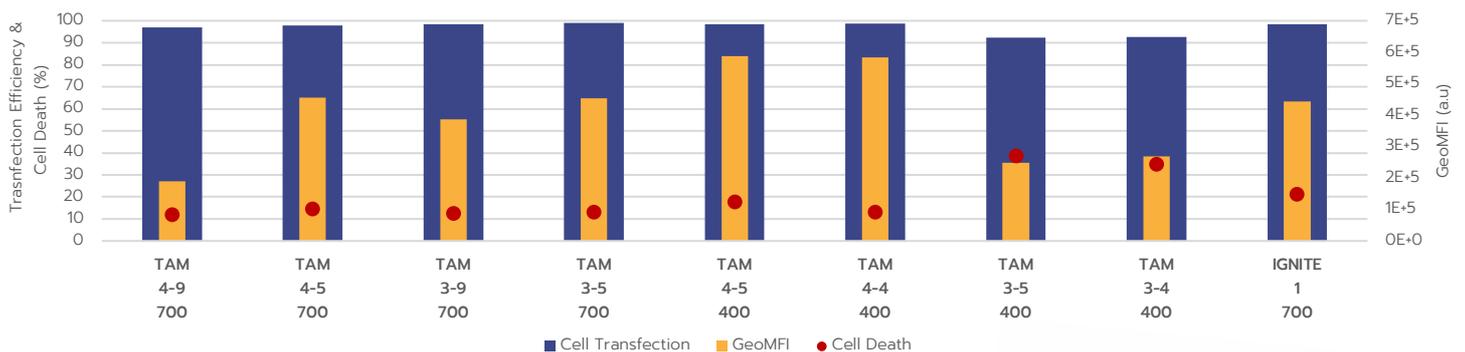
Comparison of Transcription Efficiency, Cell Viability, and Protein Expression

TFR and FRR impact on TAMARA

- **Transfection efficiency** remained consistently high (~100%) across samples.
- **Cell viability** stays high with low cytotoxicity at 700 μ L. Can decrease at lower volumes.
- **Protein expression** generally remains high across samples, though vary from formulation parameter to formulation parameter and closely align with encapsulation yield.

Systems comparisons

- **Transfection efficiency** remained consistently high (>90%) for both systems.
- **Cytotoxicity** was acceptable with both systems, though TAMARA showed lower cytotoxicity at equivalent volumes.
- **Protein expression** was strongly correlated with encapsulation yield.
- TAMARA achieved up to 30% higher **protein expression** compared to Ignite, even at lower volumes. This can be attributed to TAMARA's higher encapsulation yield and reduced cytotoxicity.



Conclusion

- **Size & PDI:** Both systems met regulatory standards (PDI < 0.2) and effectively allow for nanoparticle size control.
- **EE% & EY%:** Encapsulation efficiency is similar, but TAMARA achieves much yields over 90% at 700 μ L, compared to the Ignite's 75%.
- **In cell studies:** Both systems had similar transcription efficiencies (~100%) and low cytotoxicity, but TAMARA produced up to 30% higher protein expression.
- **RNA usage:** TAMARA's performance at 400 μ L and 700 μ L matched Ignite's 700 μ L, meaning near half the reagent use.
- **Cost-effectiveness:** TAMARA used only two reusable chips, demonstrating superior cost-efficiency.
- **Scalability:** Formulation with TAMARA can be brought from 400 μ L to 30 mL with identical performances.

contact@insidetx.com
insidetx.com



Read the full Application Note