

Controlling LNP size and PDI with microfluidic mixing

How total flow rate and flow-rate ratio set particle size, with a worked design-of-experiments example

Application note AN-01 · October 2026

Principle

In a microfluidic mixer, lipids dissolved in ethanol meet an acidic aqueous stream. As ethanol polarity rises, lipids precipitate and assemble around the nucleic acid. Faster mixing gives smaller, more uniform particles. The two main controls are total flow rate (TFR) and the aqueous:ethanol flow-rate ratio (FRR).

Materials

- Lipid mix in ethanol (ionisable lipid : DSPC : cholesterol : PEG-lipid, e.g. 50:10:38.5:1.5 mol %)
- mRNA in 25–50 mM citrate or acetate buffer, pH 4
- Staggered herringbone microfluidic chip and syringe or pressure pumps
- Dialysis cassettes or TFF unit; DLS instrument

Method

- Prime both channels and discard the first 0.2 mL of output.
- Run a 3 × 3 design: TFR 4, 8 and 12 mL/min; FRR 2:1, 3:1 and 4:1.
- Dilute output at least 1:1 in buffer immediately, then dialyse against PBS pH 7.4.
- Measure Z-average and PDI in triplicate at 25 °C.

Expected trends

Change	Effect on size	Effect on PDI
Increase TFR	Decreases, then plateaus	Usually decreases
Increase FRR (more aqueous)	Decreases	Decreases until ~3:1–4:1
Increase PEG-lipid mol %	Decreases	Minor change
Raise lipid concentration	Increases slightly	May increase

Acceptance criteria (platform)

Z-average within ± 10 nm of target; PDI < 0.15 for screening and < 0.09 for release; encapsulation $\geq 90\%$ (see AN-02).