

Measuring mRNA encapsulation efficiency with a RiboGreen assay

A plate-based protocol with detergent lysis, standard curves and common sources of error

Application note AN-02 · October 2026

Principle

RiboGreen dye fluoresces strongly when bound to RNA but cannot reach RNA inside intact particles. Measuring fluorescence with and without a detergent that disrupts the particle gives free and total RNA. Encapsulation efficiency (EE) = $(\text{total} - \text{free}) / \text{total} \times 100\%$.

Materials

- Quant-iT RiboGreen reagent and 1× TE buffer (RNase-free)
- 2% Triton X-100 in TE
- RNA standard matching your payload length
- Black 96-well plate; plate reader (ex 480 nm / em 520 nm)

Method

- Prepare a standard curve from 0 to 2 µg/mL RNA in TE and in TE + 1% Triton (detergent changes the signal slightly).
- Dilute the LNP sample to fall within the curve. Plate 50 µL into TE wells and 50 µL into TE + Triton wells, in triplicate.
- Incubate 10 min at 37 °C to lyse particles in the Triton wells.
- Add 50 µL diluted RiboGreen to all wells, protect from light for 5 min and read.
- Calculate free RNA from TE wells (TE curve) and total RNA from Triton wells (Triton curve).

Troubleshooting

Observation	Likely cause	Fix
EE above 100%	Using one standard curve for both conditions	Run matched curves with and without Triton
Low total RNA recovery	Incomplete lysis	Extend incubation or raise Triton to 1%
High variability	Signal outside linear range	Re-dilute; keep reads below top standard

Acceptance criterion (platform)

EE $\geq$ 90% for release; total RNA within $\pm 10\%$ of the theoretical concentration.