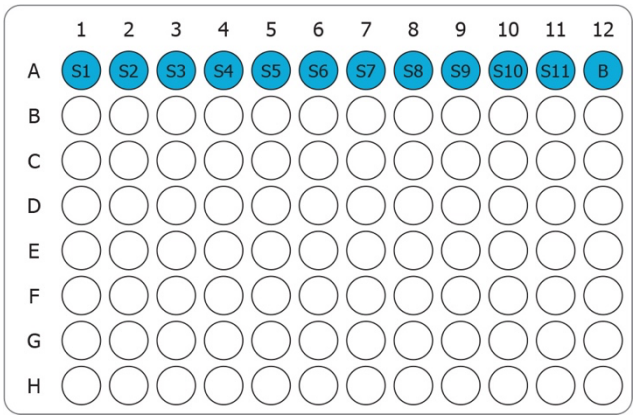
iQuant™ RiboGreen Assay Protocol

The following protocol is used to determine the encapsulation efficiency for RNA-LNPs.

Encapsulation Efficiency Assay Protocol for RNA-LNPs

Preparation of Sample Stock Solutions

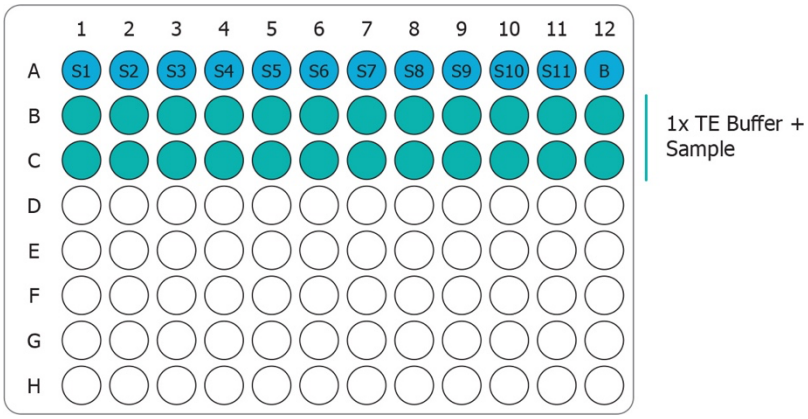
1. Transfer 100 mL of RNA buffer (component B) to a plastic container, add 2 mL of Triton X-100. Vigorously vortex for 30 seconds to mix. This solution is labeled as Triton Buffer.
2. Pour the RNA buffer and Triton buffer in separate pipette basins.
3. In the top row of the 96-well plate (Row A), add 15 µL of sample to these wells (S1- S11). Add 15 µL of PBS to the blank well (B).
4. Using a multi-channel pipette, add RNA buffer to Row A to make up the volume to 250 µL. Pipette to mix.



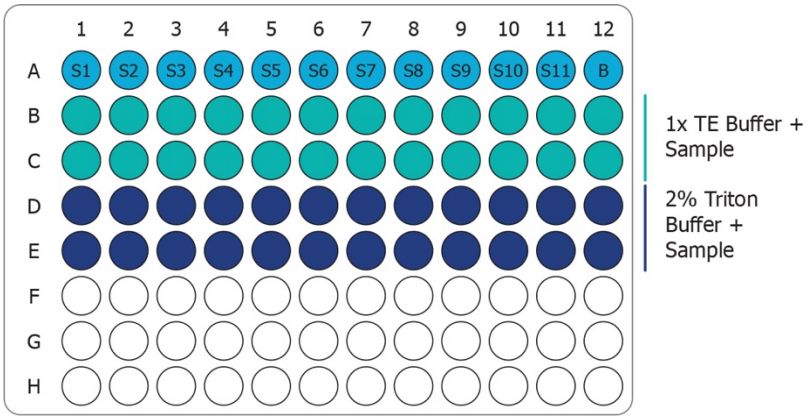
RNA-LNP Sample Setup

This assay is run in duplicate. It is recommended that liquid handling be done using a multi-channel pipette.

1. Add 50 µL of RNA buffer to the two wells directly below each sample (Rows B and C).
2. Add 50 µL of sample stock solution from Row A into the wells in Row B and C.



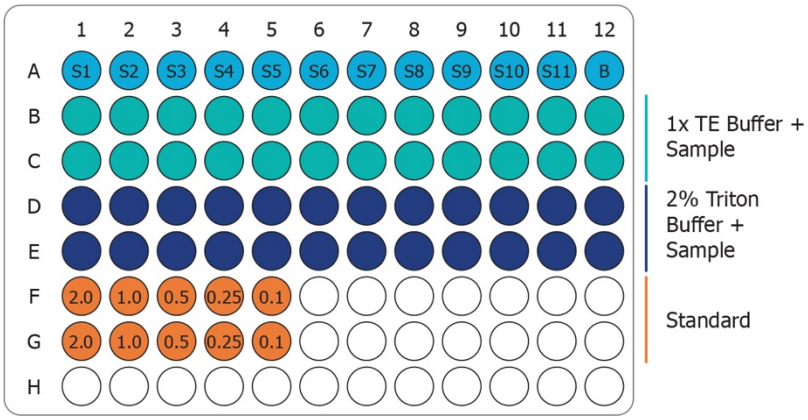
3. Add 50 µL of Triton buffer to the wells in Rows D and E below each sample.
4. Add 50 µL of sample stock solution from Row A into the wells in Rows D and E.



RNA Standard Curve Setup

1. Setup a standard curve (in duplicate in rows F and G) as shown in the table below using the RNA Stock (20 ng/µL RNA), RNA Buffer, and Triton Buffer.

Volume of 20 ng/µL RNA stock	Volume of RNA buffer	Volume of Triton buffer	Final RNA concentration
20 µL	30 µL	50 µL	2 ng/µL
10 µL	40 µL	50 µL	1 ng/µL
5 µL	45 µL	50 µL	0.5 ng/µL
2.5 µL	47.5 µL	50 µL	0.25 ng/µL
1 µL	49 µL	50 µL	0.1 ng/µL



2. Once samples and standard curve are plated, incubate the plate at 37°C for 10 min to lyse the RNA-LNP in the presence of Triton buffer.

Preparation of RiboGreen Solution

1. Sum the total number of sample wells and standard curve wells. Add four to this number, and multiply the total by 100. This is the total volume, in µl, of Ribogreen Solution needed for this assay.
2. In a 15 mL RNase Free Falcon Tube, dilute the Ribogreen Reagent 1:100 into RNA buffer to the total volume calculated in the previous step.

   Note: For example, if 3000 µl of Ribogreen Solution is needed, add 30 µl of RiboGreen Reagent to 2970 µl of RNA buffer.
3. Vortex the RiboGreen Solution for 5 seconds to mix.

Addition of RiboGreen Solution and Sample Reading

1. Remove 96-well plate from 37°C incubator.
2. Add 100 µl of RiboGreen Solution to each well.
3. Pop any bubbles with a needle.
4. Read using fluorescent plate reader with excitation and emission wavelength at 480/520nm.

Sample Analysis

Using work sheet to generate standard curve, and calculate the RNA concentration and encapsulation efficiency.