BrightQuant™ R - Nanoparticle RNA Encapsulation Efficiency (EE%) Assay Kit

Product Family: BrightQuant™ R EE% Assays (HLXBQ-R-EE Series)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available via info@helix-biotech.com

Product Description

BrightQuant™ RNA Encapsulation Efficiency (EE%) Assay Kits provide a rapid, sensitive, and convenient fluorescence-based method for determination of free RNA, total RNA, encapsulated RNA, and encapsulation efficiency (EE%) in lipid nanoparticles (LNPs), liposomes, polymer nanoparticles, and related nucleic acid delivery systems.

The assay utilizes BrightQuant™ R fluorescent RNA quantification reagent in combination with matrix-matched standard curves prepared in TE Buffer and BrightQuant™ Omni™ NP Digest Buffer. The workflow enables accurate determination of RNA encapsulation efficiency without specialized instrumentation beyond a standard fluorescence plate reader.

Applications include:

- Lipid nanoparticle (LNP) characterization
- Process development and formulation optimization
- Encapsulation efficiency determination
- Stability studies
- Manufacturing process monitoring
- High-throughput screening workflows

Dynamic Range and Sensitivity

BrightQuant™ R reagent delivers broad dynamic range and low detection limits using simple mix-and-read protocols with common microplate readers. Upon binding nucleic acids, BrightQuant™ reagents exhibit excitation and emission maxima near 500 nm and 525 nm, respectively, and are readily detected using standard fluorescein filter sets.

BrightQuant™ R RNA Reagent

- EE% Assays: Linear detection from 100 ng/mL to 2 µg/mL RNA for both Free and Total RNA

BrightQuant™ R reagent is compatible with most standard microplate readers using fluorescein filter sets (excitation 485 ± 10 nm / emission 530 ± 12.5 nm).

Interference and Compatibility

BrightQuant™ reagents maintain linearity in the presence of common lipid (LNP) and polymer nanoparticle formulation components and excipients, including:

- Lipids (ionizable/cationic lipids, phospholipids, cholesterol) and polymers (PEGs, copolymers)
- Salts, sugars, buffers
- Detergents (such as Triton X-100, Tween-20)
- Ethanol and chloroform

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EE% Assay Principle

The BrightQuant™ EE assay measures both Free RNA and Total RNA using two parallel analytical workflows.

Free RNA Measurement

RNA-LNP samples are diluted in TE Buffer and analyzed directly to quantify unencapsulated, Free RNA.

Total RNA Measurement

RNA-LNP samples are treated with BrightQuant™ Omni™ NP Digest Buffer to disrupt nanoparticles and release encapsulated RNA prior to analysis. This allows for measurement of the Total RNA concentration.

Encapsulated RNA

Encapsulated RNA is determined by subtracting Free RNA from Total RNA.

$$\text{Encapsulated RNA} = \text{Total RNA} - \text{Free RNA}$$

Encapsulation Efficiency (EE%)

Encapsulation efficiency (EE%) is determined by dividing the Encapsulated RNA by the Total RNA (expressed as a percentage).

$$EE\% = (\text{Encapsulated RNA} \div \text{Total RNA}) \times 100\%$$

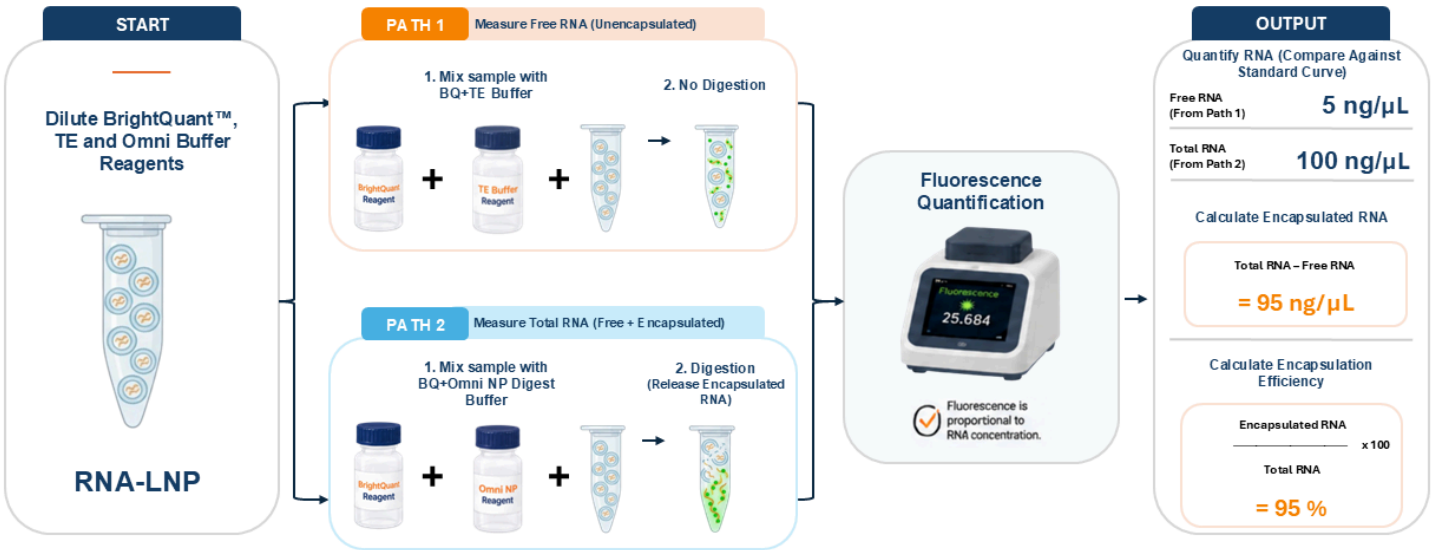

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Table 1. Contents and storage

Component	BrightQuant™ R RNA Reagent, 250µL	BrightQuant™ R RNA Reagent, 1mL	BrightQuant™ R RNA Kit	Description	Storage
BrightQuant™ R RNA Reagent	250µL	1mL	1mL	Solution in DMSO	2°C to 8°C*
20X TE Buffer	N/A	N/A	25mL	TE Buffer for general nucleic acid quantitation	2°C to 8°C*
20x BrightQuant™ Omni™ Nanoparticle Digest Buffer	N/A	N/A	25mL	Proprietary buffer optimized for nanoparticle digest and EE% assays	2°C to 8°C*
RNA standard (1kb)	N/A	N/A	5 x 100µL	100 µg/mL 1kb mRNA	≤ -20°C or -80°C **
Approximate fluorescence excitation/emission maxima: 500/525 nm, bound to nucleic acid.					

When stored as directed, products are stable for at least 6 months.
*For long-term storage, store at ≤–20°C. Protect BrightQuant™ R Reagent from prolonged light exposure.
**For maximum stability, store at ≤ -20°C or -80°C. Short term storage of thawed standard at 2°C to 8°C is acceptable. Avoid repeated freeze-thaw cycles of RNA standards.
Disclaimer: This material is intended for research use only and is not intended for human use.

Additional Reagents/Supplies Required

- Nuclease-free pipettors and tips
- Nuclease-free water
- Microplates for Fluorescence-based Assays, 96-well
- Microcentrifuge tubes or centrifuge tubes
- Pipette reservoir

Additional Equipment Required

- Fluorescent Plate Reader (i.e. BioTek™ Synergy™ Plate Reader)
- Micropipettes (10, 20, 200, 1000, 5000 µL)
- Multi-channel pipette (300µL)

Prepare the assay buffers

Use commercially available nuclease-free water or molecular biology grade RNase-free water. Store buffers at 2°C-8°C and use them within 2 weeks.

TE Buffer: To prepare 1X TE Buffer, dilute the supplied 20X TE Buffer 20-fold (1:19) with nuclease-free water. For example, using a 50mL conical tube labeled "TE Buffer", dilute 2mL of 20X TE Buffer into 38mL nuclease-free water. Mix by inverting 3-5 times or vortexing.

BrightQuant™ Omni™ NP Digest Buffer: To prepare 1X Omni™ NP Digest Buffer, dilute the supplied 20X Omni™ NP Digest Buffer 20-fold (1:19) with nuclease-free water. For example, using a 50mL conical tube labeled "Digest Buffer", dilute 2mL of 20X Omni™ NP Digest Buffer into 38mL nuclease-free water. Mix by inverting gently at least 5 times (vortexing is not recommended as it may induce formation of bubbles).

BrightQuant™ R Reagent Working Solution Preparation

1. Allow the BrightQuant™ R RNA Reagent to equilibrate to room temperature before opening the vial.
2. Prepare the working solution by diluting the reagent 200-fold (1:199) into TE buffer. For example, using a 15mL conical tube, dilute 50 µL reagent into 9.95 mL of 1x TE Buffer to prepare for 100 samples. Mix by inverting 3-5 times or vortexing briefly.
3. For 200 µL microplate assays, use 100 µL of working solution per well.

IMPORTANT! Handling Notes

- Warm reagent to room temperature before use. Opening cold DMSO solutions may cause condensation and reduce efficacy.
- Store the DMSO stock with desiccant when not in use.
- Prepare the working solution in sterile polypropylene plasticware, not glass, to avoid dye adsorption.
- Protect from light to prevent photodegradation.
- Use the working solution within a few hours of preparation for best results.

RNA standard curve preparation

Two separate standard curves are strongly recommended. One set of standards is prepared using TE Buffer as the diluent for quantifying Free RNA in nanoparticle samples diluted in TE Buffer. A second set of standards is prepared using BrightQuant™ Omni™ NP Digest Buffer as the diluent for quantifying Total RNA in nanoparticle samples diluted in Omni™ NP Digest Buffer.

Standards are prepared in sufficient volume below for multiplate uses and can be stored at 2°C to 8°C for up to 2 weeks (minimize freeze/thaws). Volumes may be adjusted up or down to produce larger/smaller volumes of each standard.

> 💡 Note: While the supplied RNA standard is recommended, you may substitute with a purified RNA sample that closely matches the type of RNA in your assay.
>
> ⚠️ The RNA solution used to prepare the standard curve is ideally treated identically to experimental samples.

Free RNA Standard Curve:

Table 2. Protocol for preparing Free RNA standard curve in TE Buffer.

Std #	Volume of 1X TE Buffer	Volume of 100 µg/mL RNA standard	Volume of Previous Level	Final RNA concentration
1	4900 µL	100 µL	0 µL	2 µg/mL
2	2500 µL	0 µL	2500 µL	1 µg/mL
3	2500 µL	0 µL	2500 µL	500 ng/mL
4	2500 µL	0 µL	2500 µL	250 ng/mL
5	2500 µL	0 µL	2500 µL	125 ng/mL
6	5000 µL	0 µL	0 µL	Blank (0 ng/mL)

Total RNA Standard Curve

Table 3. Protocol for preparing Total RNA standard curve in BrightQuant™ Omni™ NP Digest Buffer.

Std #	Volume of 1X Omni NP Digest Buffer	Volume of 100 µg/mL RNA standard	Volume of Previous Level	Final RNA concentration
1	4900 µL	100 µL	0 µL	2 µg/mL
2	2500 µL	0 µL	2500 µL	1 µg/mL
3	2500 µL	0 µL	2500 µL	500 ng/mL
4	2500 µL	0 µL	2500 µL	250 ng/mL
5	2500 µL	0 µL	2500 µL	125 ng/mL
6	5000 µL	0 µL	0 µL	Blank (0 ng/mL)

Notes:

- For best results, adjust the **instrument gain** so that the highest RNA concentration sample produces a signal near the upper limit of the reader's detection range (some instruments describe this setting as "scale to high wells" where you would then select the wells with the highest standard concentrations for use in gain threshold determination).
- For low-range assays, a higher gain setting may improve sensitivity.
- To minimize photobleaching, keep the read time consistent across all samples.

Nanoparticle Sample Preparation

Important

> ⚠️ Free RNA and Total RNA samples frequently require different dilution factors and should be diluted independently.
>
> ⚠️ Free RNA samples should be diluted with TE Buffer. Free RNA samples should be quantified against standards prepared in TE Buffer.
>
> ⚠️ Total RNA samples should be diluted with Omni™ NP Digest Buffer. Total RNA samples should be quantified against standards prepared in Omni™ NP Digest Buffer.

Recommended Workflow

For highest accuracy and reproducibility, prepare standards and sample dilutions in microcentrifuge tubes prior to plate loading.

Advantages:

- Reduced pipetting error
- Improved reproducibility
- Easier duplicate and triplicate preparation
- Simplified dilution calculations
- Compatible with robotic and automated liquid handling systems

This workflow is recommended for formulation development, process development, and routine analytical testing. See additional guidelines and examples for sample dilution factors below.

Free RNA Samples:

Nanoparticle Sample Dilution for Free RNA. Dilute each experimental RNA-LNP sample in 1X TE Buffer such that the expected RNA concentration falls within the standard curve range. For a typical 96-well plate format, prepare at least 300 µL of diluted sample to allow measurement in triplicate (3 × 100 µL).

For RNA-LNP formulations containing approximately 100-1,000 µg/mL total RNA and less than 10% free RNA, a 50X dilution is often an appropriate starting point for Free RNA analysis .

Total RNA Samples:

Nanoparticle Sample Dilution for Total RNA. Dilute each experimental RNA-LNP sample in 1X BrightQuant™ Omni™ NP Digest Buffer such that the expected RNA concentration falls within the standard curve range. For a typical 96-well plate format, prepare at least 300 µL of diluted sample to allow measurement in triplicate (3 × 100 µL).

For RNA-LNP formulations containing approximately 100-400 µg/mL total RNA, a 200X dilution is often an appropriate starting point. Samples above approximately 400 µg/mL total RNA may require 1,000X dilution or greater.

Incubate diluted samples for 15 minutes at room temperature to allow nanoparticle disruption and release of encapsulated RNA. Cover plate with a plate lid or aluminum foil while incubating.

Free RNA Samples (Example Starting Conditions)

For RNA-LNP formulations containing approximately 100-1,000 µg/mL total RNA and less than 10% free RNA, a 50X dilution is often an appropriate starting point.

Table 4. Sample Dilution in 1X TE Buffer

Dilution Factor	Sample (µL)	1X TE Buffer (µL)	From
50X	10	490	Sample

Total RNA Samples (Example Starting Conditions)

For RNA-LNP formulations containing approximately 100-400 µg/mL total RNA, a 200X dilution is often appropriate. Higher concentration samples may require 1,000X dilution or greater.

Table 5a. Initial 10X Sample Dilution in 1X BrightQuant™ Omni™ NP Digest Buffer

Dilution Factor	Sample (µL)	1x Omni™ NP Digest Buffer (µL)	From
10X	10	90	Sample

Table 5b. Serial Dilution for Total RNA Analysis

Dilution Factor	Sample (µL)	1x Omni™ NP Digest Buffer (µL)	From
200X	20	380	10X
1000X	10	990	10X

Typical Starting Dilutions

For RNA-LNP formulations containing:

- 100-400 µg/mL RNA → Start with 50X (Free) and 200X (Total)
- 400-1,000 µg/mL RNA → Start with 50X (Free) and 1,000X (Total)
- >1,000 µg/mL RNA → Higher dilution factors may be required

These are recommended starting conditions only. Additional optimization may be required depending on encapsulation efficiency and formulation composition.

If sample fluorescence exceeds the highest standard curve level, repeat using a greater dilution.

Plate Setup

Load 100 µL of each standard and each diluted sample into three replicate wells.

Example plate layouts are provided below. Standards and samples may be arranged differently as needed. The layouts shown assume that each standard and diluted sample is analyzed in triplicate wells (100 µL per well).

For highest accuracy, independent sample dilutions may be prepared in duplicate or triplicate prior to plating (for example, S1-1 and S1-2). Each dilution is then loaded into three replicate wells. Additional samples may be placed in any unused wells. The layouts below illustrate common configurations for analyzing one to four nanoparticle samples (S1, S2, S3, S4).

Plate Layout Option 1: Single NP Sample, Single Independent Dilutions, Three Replicate Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	TE STD1	TE STD1	TE STD1	Digest STD1	Digest STD1	Digest STD1	TE S1-1	TE S1-1	TE S1-1	Digest S1-1	Digest S1-1	Digest S1-1
B	TE STD2	TE STD2	TE STD2	Digest STD2	Digest STD2	Digest STD2						
C	TE STD3	TE STD3	TE STD3	Digest STD3	Digest STD3	Digest STD3						
D	TE STD4	TE STD4	TE STD4	Digest STD4	Digest STD4	Digest STD4						
E	TE STD5	TE STD5	TE STD5	Digest STD5	Digest STD5	Digest STD5						
F	TE STD6	TE STD6	TE STD6	Digest STD6	Digest STD6	Digest STD6						
G												
H												

Plate Layout Option 2: 1-4 NP Samples, Duplicate Independent Dilutions, Three Replicate Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	TE STD1	TE STD1	TE STD1	Digest STD1	Digest STD1	Digest STD1	TE S1-1	TE S1-1	TE S1-1	Digest S1-1	Digest S1-1	Digest S1-1
B	TE STD2	TE STD2	TE STD2	Digest STD2	Digest STD2	Digest STD2	TE S1-2	TE S1-2	TE S1-2	Digest S1-2	Digest S1-2	Digest S1-2
C	TE STD3	TE STD3	TE STD3	Digest STD3	Digest STD3	Digest STD3	TE S2-1	TE S2-1	TE S2-1	Digest S2-1	Digest S2-1	Digest S2-1
D	TE STD4	TE STD4	TE STD4	Digest STD4	Digest STD4	Digest STD4	TE S2-2	TE S2-2	TE S2-2	Digest S2-2	Digest S2-2	Digest S2-2
E	TE STD5	TE STD5	TE STD5	Digest STD5	Digest STD5	Digest STD5	TE S3-1	TE S3-1	TE S3-1	Digest S3-1	Digest S3-1	Digest S3-1
F	TE STD6	TE STD6	TE STD6	Digest STD6	Digest STD6	Digest STD6	TE S3-2	TE S3-2	TE S3-2	Digest S3-2	Digest S3-2	Digest S3-2
G							TE S4-1	TE S4-1	TE S4-1	Digest S4-1	Digest S4-1	Digest S4-1
H							TE S4-2	TE S4-2	TE S4-2	Digest S4-2	Digest S4-2	Digest S4-2

Assay Procedure

1. Add 100 µL of each standard or diluted sample to the appropriate microplate wells.
2. Add 100 µL of BrightQuant™ R Working Solution to each well.
3. Mix gently by pipetting or brief plate shaking. Avoid bubbles.
4. Incubate for 5 minutes at room temperature, protected from light.
5. Measure fluorescence using the same instrument settings used for the standard curves.

Data Analysis

1. Subtract blank values and determine Free RNA and Total RNA concentrations for each well from the appropriate matrix-matched standard curve.
    a. Determine Free RNA concentration from the TE Buffer standard curve.
    b. Determine Total RNA concentration from the Omni™ NP Digest Buffer standard curve.
2. Encapsulated RNA = Total RNA − Free RNA
3. EE% = (Encapsulated RNA ÷ Total RNA) × 100%

Plate Reader Settings

Parameter	Setting
Excitation	485 ± 10 nm
Emission	530 ± 12.5 nm
Read Mode	Endpoint Fluorescence
Gain	Auto or optimized from highest standard wells
Plate Type	Black 96-well (or other opaque 96-well)

Technical Support

For technical assistance, application support, or automation workflows, contact:

Helix Biotech, Inc.
Knoxville, Tennessee, USA

support@helix-biotech.com
www.helixbiotech.com

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Frequently Asked Questions

How much should I dilute my sample?
Dilute samples such that measured fluorescence falls within the standard curve range. Additional dilution may be required for highly concentrated formulations.

Should Free RNA and Total RNA samples be diluted equally?
Free RNA and Total RNA samples often require different dilution factors. Use dilution factors that place each measurement within its matrix-matched standard curve range. See pages 6-7.

What is the purpose of the 20X TE Buffer?
TE Buffer is used for sample dilution, standard preparation, and Free RNA analysis.

What is the purpose of Omni™ NP Digest Buffer?
Omni™ NP Digest Buffer disrupts nanoparticles and releases encapsulated RNA for Total RNA analysis.

Why are separate standard curves required for Free RNA and Total RNA?
Free RNA samples are measured in TE Buffer while Total RNA samples are measured in Omni™ NP Digest Buffer. Matrix-matched standards compensate for differences in fluorescence response between these conditions and improve assay accuracy.

Can I reuse diluted standards?
Fresh standards are recommended when practical. Prepared standards may be stored at 2°C to 8°C for up to 2 weeks.

Can I use the supplied BrightQuant™ Ready-To-Use Standard Sets?

Yes. BrightQuant™ Ready-To-Use Standard Sets are available separately and eliminate the need for standard curve preparation.

Can I add lipids or ethanol to standards?
Matrix matching may improve accuracy when significant lipid, solvent, or formulation components are present.

Can I run this assay on a liquid handler?
Yes. BrightQuant™ EE assays are compatible with automated liquid handling systems including Opentrons®, Hamilton®, Tecan®, and similar platforms.

Can I use a fluorimeter or cuvette-based instrument?
Yes. Assay volumes may be scaled as needed for fluorimeters, cuvettes, microtubes, and other fluorescence-based measurement systems.

What type of plate should I use?
Black fluorescence microplates, or other opaque microplates, are recommended for optimal sensitivity and reduced background signal.

What EE% values are typically expected?
Well-optimized RNA-LNP formulations commonly exhibit encapsulation efficiencies greater than 90%.