BrightQuant™ R - RNA Assay Kit/Reagent

Product Family: BrightQuant™ R (HLXBQ-R 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™ Fluorescent Nucleic Acid Quantification Reagents are high-sensitivity, easy-to-use fluorescent dyes for accurate quantification of RNA, DNA, and oligonucleotides in solution, with special applications for measuring encapsulation efficiency in lipid nanoparticles (LNPs), polymer nanoparticles, and liposomes.

Each BrightQuant™ reagent is formulated to provide maximum linearity, reproducibility, and convenience, across a wide range of concentrations and sample types. BrightQuant™ R is optimized for RNA quantification, BrightQuant™ D for double-stranded DNA (dsDNA), and BrightQuant™ O for oligonucleotides and single-stranded DNA.

BrightQuant™ reagents are especially useful for:

- Quantifying mRNA yields from IVT reactions
- Measuring nucleic acid concentrations before RT-PCR, NGS, or cloning
- High-throughput microplate-based quantification workflows (Typical 96-well microplate workflow. BrightQuant™ assays may also be adapted to microtube, cuvette, fluorimeter, and automated liquid handling formats.)

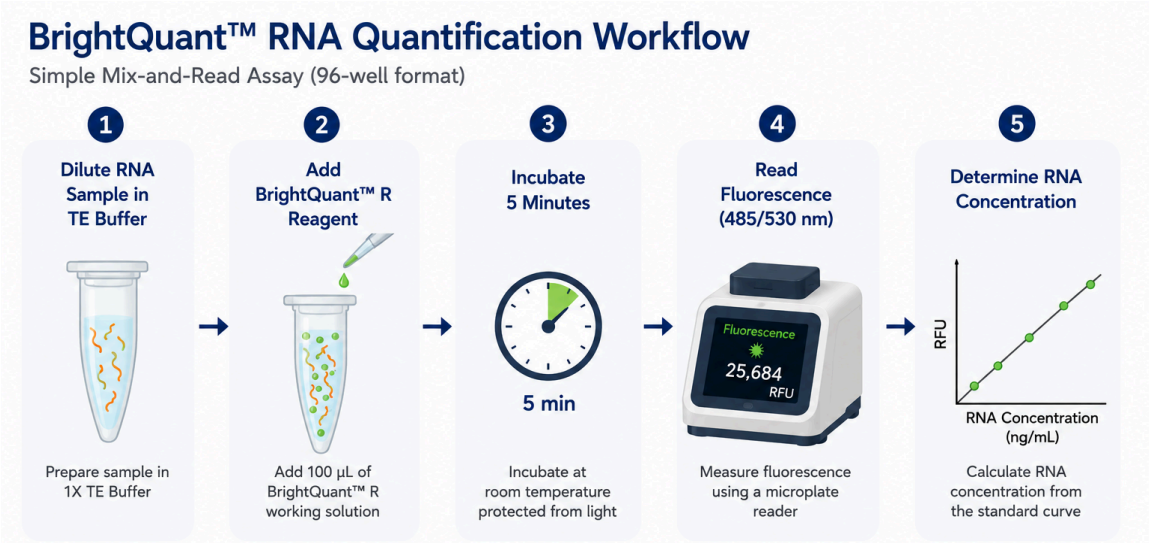
Dynamic Range and Sensitivity

BrightQuant™ assays deliver 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

- High-range mode: Linear detection from 20 ng/mL to 1 µg/mL RNA
- Low-range mode: Detects as little as 1 ng/mL RNA (200 pg per 200 µL assay)

Each assay can be tuned for high or low sensitivity by adjusting the dye dilution, and 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 nucleic acid preparation contaminants, including:

- Free nucleotides
- Detergents (such as Triton X-100, Tween-20)
- Urea, salts, ethanol, and chloroform
- Bovine serum albumin (BSA) and other proteins

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

Prepare the assay buffer

Use commercially available nuclease-free water or molecular biology grade RNase-free water.

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™ R Reagent Working Solution Preparation

1. Allow the BrightQuant™ R Reagent to equilibrate to room temperature before opening the vial.
2. Prepare the working solution by diluting the Reagent into TE buffer as follows:
    - High-range assay: dilute 200-fold (1:199)
      (e.g., 50 µL reagent + 9.95 mL TE for 100 samples)
    - Low-range assay: dilute 2,000-fold (1:1,999)
      (e.g., 5 µL reagent + 9.995 mL TE for 100 samples)
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.

Prepare the RNA standard curve

1. Prepare a 2 µg/mL working RNA standard by diluting the supplied 100 µg/mL RNA standard 50-fold (1:49, 4 µL standard + 196 µL TE buffer). This volume is sufficient for a complete standard curve.

   💡 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.

2. For the **high-range Assay** (20 ng/mL – 1 µg/mL), Prepare a serial dilution directly from the 2 µg/mL RNA solution according to the concentrations listed in Table 2. For the **low-range Assay** (1 ng/mL – 50 ng/mL), First, dilute the 2 µg/mL RNA solution 20-fold in TE to make a 100 ng/mL stock. Then Prepare a dilution series using this 100 ng/mL stock, as described in Table 3.

3. Add Reagent and Incubate. Add 100 µL of the appropriate diluted BrightQuant™ R Reagent working solution (high-range or low-range) to each well. Mix and incubate 5 minutes at room temperature, protected from light.
4. Measure Fluorescence. Measure fluorescence using a microplate reader with standard fluorescein settings (excitation: ~480 nm, emission: ~520 nm)

5. Analyze Data. Subtract the fluorescence of the reagent blank from each sample's reading to obtain corrected fluorescence values. Use the corrected data to generate a standard curve of fluorescence vs. RNA concentration.

Table 2. Protocol for preparing a high-range standard curve.

Volume of TE buffer	Volume of 2 µg/mL RNA stock	Volume of Diluted High-Range Reagent Working Solution	Final RNA concentration in the assay
0 µL	100 µL	100 µL	1 µg/mL
50 µL	50 µL	100 µL	500 ng/mL
90 µL	10 µL	100 µL	100 ng/mL
95 µL	5 µL	100 µL	50 ng/mL
98 µL	2 µL	100 µL	20 ng/mL
100 µL	0 µL	100 µL	blank

Table 3. Protocol for preparing a low-range standard curve.

Volume of TE buffer	Volume of 100 ng/mL RNA stock	Volume of Diluted Low-Range Reagent Working Solution	Final RNA concentration in the assay
0 µL	100 µL	100 µL	50 ng/mL
50 µL	50 µL	100 µL	25 ng/mL
80 µL	20 µL	100 µL	10 ng/mL
90 µL	10 µL	100 µL	5 ng/mL
98 µL	2 µL	100 µL	1 ng/mL
100 µL	0 µL	100 µL	blank

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.

Analyze samples

1. Sample Dilution. Dilute each experimental RNA sample in 1X TE Buffer such that the expected RNA concentration falls within the appropriate standard curve range (high-range or low-range). For a typical 96-well plate format, prepare 300 µL or more to allow measurement in triplicate (3 x 100 µL).

💡 Notes:

- Higher dilution can help minimize interference from contaminants.
- Avoid using very small sample volumes, as they can be difficult to pipette accurately.
- To ensure reliable comparisons, keep contaminant levels consistent across all samples. Variations in salt concentration or other interfering substances can lead to inaccurate quantification. If necessary, normalize sample buffer conditions prior to the assay.

2. Add samples to the assay vessel (microplate well, microtube, cuvette, or other compatible fluorescence format). For a typical 96-well plate format, add 100 µL of diluted sample to each well for analysis.

3. Add an equal volume of diluted BrightQuant™ R Reagent working solution and Incubate (prepared earlier). For a typical 96-well plate format, add 100 µL of the diluted BrightQuant™ R Reagent working solution to each well. Gently mix and incubate for 5 minutes at room temperature, protected from light.

4. Measure Fluorescence. Measure fluorescence using the same instrument settings used to generate the standard curve. Keep the read time consistent across all wells to minimize photobleaching and ensure accurate comparison.

5. Data Correction and Quantification. Subtract the fluorescence of the reagent blank from each sample reading to obtain corrected values. Determine the RNA concentration using the standard curve prepared earlier.

6. **Optional**: Reassay for Accuracy. If needed, repeat the assay using a different sample dilution to confirm the accuracy and consistency of your quantification results.