StrandBrite™ Green Fluorimetric RNA Quantitation Kit *Optimized for Microplate Readers*

Catalog number: 17655
Unit size: 1000 Tests

Component	Storage	Amount
Component A: StrandBrite™ Green	Freeze (< -15 °C), Minimize light exposure	1 vial (0.5 mL, 200X in DMSO)
Component B: 10X Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: RNA Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (0.2 mL, 100 µg/mL)

OVERVIEW

Detecting and quantitating small amounts of RNA is extremely important for a wide variety of molecular biology procedures such as measuring yields of in vitro transcribed RNA and measuring RNA concentrations before performing Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library preparation, reverse transcription PCR, and differential display PCR. The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm. The major disadvantage of the absorbance-based method is the interferences caused by proteins, free nucleotides and other UV absorbing compounds. The use of sensitive, fluorescent nucleic acid stains alleviates this interference problem. StrandBrite™ RNA quantifying reagent is an ultrasensitive fluorescent nucleic acid stain for quantitating RNA in solution. StrandBrite™ RNA quantifying reagent can detect as little as 5 ng/mL RNA with a fluorescence microplate reader or fluorometer. Our StrandBrite™ Green Fluorimetric RNA Quantitation Kit includes our StrandBrite™ Green nucleic acid stain with an optimized and robust protocol. It provides a convenient method for quantifying RNA in solutions.

AT A GLANCE

Protocol Summary

1. Add 100 µL RNA standards or test samples
2. Add 100 µL StrandBrite Green™ working solution
3. Incubate at RT for 2-5 minutes
4. Monitor the fluorescence at Ex/Em=490/545 nm

Important The following protocol is an example for quantifying RNA with StrandBrite™ Green. Allow all the components to warm to room temperature before opening. Prevent RNase contamination of the StrandBrite™ reagent and kit components. Always use clean disposable gloves while handling all materials. Use nuclease-free water and sterile, disposable polypropylene plastic ware for reagent preparation. *Caution:* No data are available for addressing the mutagenicity or toxicity of StrandBrite™ Green RNA stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with caution as DMSO is known to facilitate the entry of organic molecules into tissues.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	545 nm
Cutoff	515 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Assay Buffer stock solution (1X)
Dilute the concentrated 10X Assay Buffer (Component B) to 1X Assay Buffer stock solution with sterile, distilled, nuclease-free water.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17655

RNA standard
Add 10 µL of 100 µg/mL RNA Standard (Component C) to 990 µL of 1X Assay Buffer to have 1 µg/mL RNA standard solution (RS7). Then perform 1:3 serial dilutions to get remainder serial dilutions (RS1 - RS6).

PREPARATION OF WORKING SOLUTION

Prepare StrandBrite™ Green working solution by making a 200-fold dilution of the concentrated DMSO solution in 1X Assay Buffer. For example, add 50 µL of StrandBrite™ Green (Component A) into 10 mL of 1X Assay Buffer. Protect the working solution from light by covering it with foil or placing it in the dark.

Note We recommend preparing this solution in a plastic container rather than glass, as the dye may adsorb to glass surfaces. For best results, use promptly, within a few hours of its preparation.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of RNA standards and test samples in a solid black 96-well microplate. SD = RNA standard (SD1 - SD7, 1 to 1000 ng/mL); BL = blank control; TS = test sample.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	100 µL	serial dilution (1 to 1000 ng/mL)
BL	100 µL	TE Buffer
TS	100 µL	sample

1. Prepare RNA standards (RS), blank controls (BL), and test samples (TS) in a 96-well solid black microplate according to the layout provided in Table 1 and Table 2. For a 384-well plate, add 25 µL of reagent per well instead of 100 µL.

2. Add 100 µL of StrandBrite™ Green working solution to each well of the RNA standard, blank control, and test samples to make the total RNA assay volume of 200 µL/well. For a 384-well plate, add 25 µL of working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 2 to 5 minutes; protect from light.

4. Monitor the fluorescence increase with a spectrofluorometer at Ex/Em = 490/545 nm (cutoff at 515 nm).

   Note To minimize photobleaching, keep the time for fluorescence measurement constant across all samples.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate RNA samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

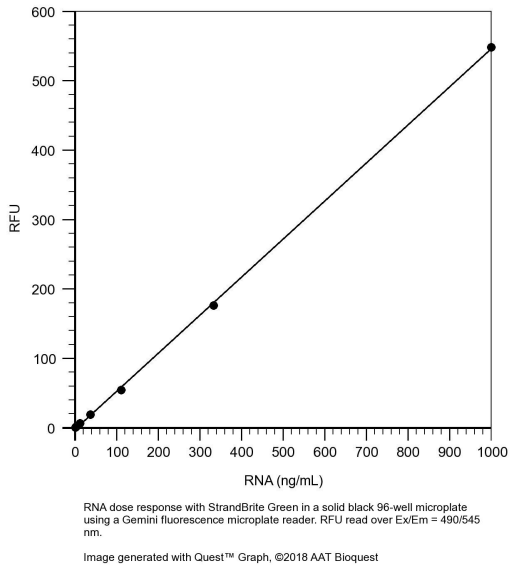

Figure 1. RNA dose response with StrandBrite Green in a solid black 96-well microplate using a Gemini fluorescence microplate reader. RFU read over Ex/Em = 490/545 nm.

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