StrandBrite™ Green RNA Quantifying Reagent *200X DMSO Solution*

Catalog number: 17610, 17611
Unit size: 1 ml, 10 ml

Component	Storage	Amount (Cat No. 17610)	Amount (Cat No. 17611)
StrandBrite™ Green RNA Quantifying Reagent	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mL)	1 bottle (10 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.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Solid black

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17610

RNA Standard
Prepare a 100 ug/mL stock solution of RNA in DEPC treated water. Prepare 2 ug/mL in TE buffer from that stock and perform 1:2 dilutions to get 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 0 ng/mL (Blank).

PREPARATION OF WORKING SOLUTION

StrandBrite™ Green working solution
Prepare an aqueous working solution of the StrandBrite™ Green by making a 200-fold dilution of the concentrated DMSO solution in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5 in DEPC treated water). For example, add 50 µL StrandBrite™ Green to 10 mL TE buffer to prepare enough working solution to assay 100 samples in a 200 µL final volume. 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.

Note For the best results, this solution should be used within a few hours of its preparation.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol is an example for quantifying RNA with StrandBrite™ Green. Allow the StrandBrite™ Green to warm to room temperature before opening the vial.

Note Always use clean disposable gloves while handling all materials to prevent RNase contamination.

Note No data are available 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.

Table 1. Layout of RNA standards and test samples in a solid black 96-well microplate. (RS = RNA Standard; BL = Blank Control; TS = Test Samples)

BL	BL	TS	TS
RS1	RS1	...	...
RS2	RS2		
RS3	RS3		
RS4	RS4		
RS5	RS5		
RS6	RS6		
RS7	RS7		

Table 2. Reagent composition for each well (Add the serially diluted RNA Standards from 15.6 to 1000 ng/mL into wells from RS1 to RS7 in duplicate)

RNA Standard	Blank Control	Test Sample
Serial Dilutions (100 µL)	TE: 100 µL	100 µL

Run RNA assay

1. 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.

   Note For a 384-well plate, add 25 µL sample and 25 µL of StrandBrite™ Green working solution per well.

2. Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.

3. Monitor the fluorescence increase with a spectrofluorometer at Ex/Em = 490/525 nm (cutoff at 515 nm).

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

4. The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those cuvettes with RNA standard or test samples. The RNA concentration of the sample is determined according to the RNA standard curve.

EXAMPLE DATA ANALYSIS AND FIGURES

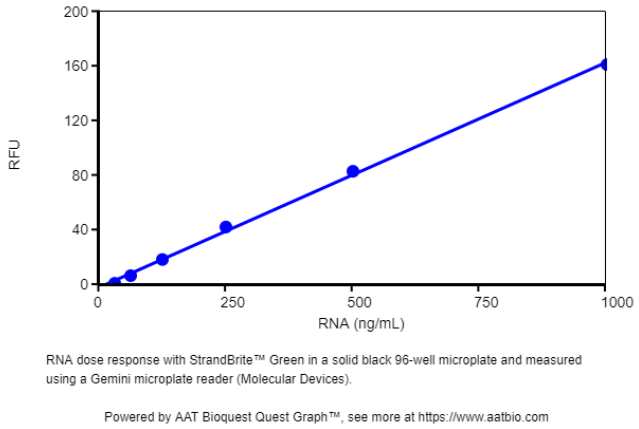

Figure 1. RNA dose response with StrandBrite™ Green in a solid black 96-well microplate and measured using a Gemini microplate reader (Molecular Devices).

DISCLAIMER

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