StrandBrite™ Green RNA Quantifying Reagent *200X DMSO Solution

Ordering Information	*Storage Conditions*
Product Number: 17610 (1 mL) and 17611 (10 mL)	Keep in -20°C. Avoid exposure to light

Biological Applications

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 as5 ng/mL RNA with a fluorescence microplate reader or fluorometer.

Assay 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 1: Always use clean disposable gloves while handling all materials to prevent RNase contamination.
Note 2: 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.

1. Preparing the StrandBrite™ Green working solution:

1.1 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 1: We recommend preparing this solution in a plastic container rather than glass, as the dye may adsorb to glass surfaces.
Note 2: For the best results, this solution should be used within a few hours of its preparation.

2. Prepare serial dilutions of RNA standard (0 to 1 µg/mL):

2.1 Prepare a 100 µg/mL stock solution of RNA in DEPC treated water.

2.2 Add 10 µL of 100 µg /mL RNA stock solution (from Step 2.1) to 490 µL of Assay buffer (Component B) to have 2 µg/mL RNA solution, and then perform 1:2 serial dilutions to get 1000, 500, 250, 125, 62.5, 31.3, 15.6, and 0 ng/mL.

2.3 Add RNA standards and RNA containing test samples into a 96-well solid black microplate as described in Tables 1 and 2.

Table 1. Layout of RNA standards and test samples in a solid black 96-well microplate*

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

**Note: RS= RNA Standards; BL=Blank Control; TS=Test Samples*

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Table 2. Reagent composition for each well*

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

**Note: Add the serially dilutions of RNA standards from 15.6 to1000 ng/mL into wells from DS1 to DS7 in duplicate.*

3. Run dsDNA assay:

3.1 Add 100 µL of StrandBrite™ Green working solution (from Step 2) to each well of the RNA standard, blank control, and test samples (see Step 3) 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.

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

3.3 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.

3.4 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.

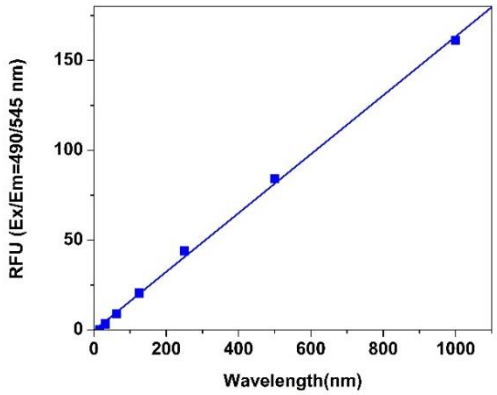

Figure 2. RNA dose response with StrandBrite™ Green in a solid black 96-well microplate and measured using a Gemini microplate reader (Molecular Devices).

References

1. Jones LJ, Yue ST, Cheung CY, Singer VL. (1996) RNA quantitation by fluorescence-based solution assay: RiboGreen reagent characterization. Anal Biochem 15, 265(2):368-74.
2. Le Pecq JB, Paoletti C. (1993) A new fluorometric method for RNA and DNA determination. Anal Biochem 17(1):100-7