StrandBrite™ Green Fluorimetric RNA Quantitation Kit
Optimized for fluorescent microplate readers

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 17655 (1000 Assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

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 1.4 ng/mL RNA with a fluorescence microplate reader or spectrofluorometer. 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.

Kit Components

Components	Amount
Component A: StrandBrite™ Green	0.5 mL (200X in DMSO)
Component B: 10X Assay Buffer	20 mL
Component C: Ribosomal RNA Standard	0.2 mL (100 μg/mL)

Sample Protocol for One 96-well Plate

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.

1. Preparing 1X assay buffer

Prepare a 1X assay buffer by diluting the concentrated buffer 20X (Component B) with sterile, distilled, nuclease-free water.

2. Preparing StrandBrite™ Green 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 (from Step 1). 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 best results, this solution should be used within a few hours of its preparation.

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

3.1 Add 10 μL of 100 μg/mL RNA stock solution (Component C) to 990 μL of Assay buffer (Component B) to have 1 μg/mL RNA solution, and then perform 1:3 serial dilutions to get approximately 1000, 300, 100, 30, 10, 3, 1 and 0 ng/mL.

Note: Unused Ribosomal RNA Standard (Component C) should be divided into single use aliquots in nuclease-free plastic vials and stored at -20°C.

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

©2015 by AAT Bioquest®, Inc., 520 Mercury Drive, Sunnyvale, CA 94085. Tel: 408-733-1055
Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

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*

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 1.4 to 1000 ng/mL into wells from RS1 to RS7 in duplicate.*

4. Run RNA assay:

4.1 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.

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

4.3 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 for all samples.

4.4 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 are determined according to the RNA standard curve.

Data Analysis

The fluorescence reading in blank cuvette (with assay buffer only) is used as a control, and is subtracted from the values of those cuvettes with the RNA standards or test samples. A RNA standard curve is shown in Figure 1. Calculate the RNA concentration of the samples according to the RNA standard curve.

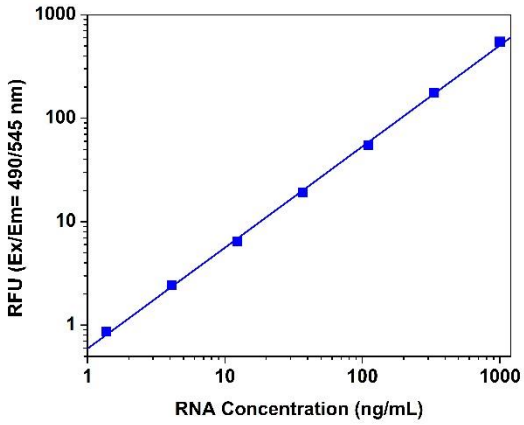

Figure 1. RNA dose response with StrandBrite™ Green in cuvettes and measured using a Gemini microplate reader (Molecular Devices).

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.

©2015 by AAT Bioquest®, Inc., 520 Mercury Drive, Sunnyvale, CA 94085. Tel: 408-733-1055
Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055