StrandBrite™ Green Fluorimetric RNA Quantitation Kit

High Selectivity

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 17657 (100 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 major challenge of current RNA quantitation assays is the interferences caused by DNA. In order to address the poor selectivity of small molecular probes for RNA, a sensitive, fluorogenic RNA probe with high selectivity was developed. StrandBrite™ RNA Green is an ultrasensitive fluorescent nucleic acid stain for quantitating RNA in solution. Compared to other RNA probes widely used in the laboratory, StrandBrite™ RNA Green shows a much larger binding affinity to RNA than DNA (e.g, duplex DNA and single-stranded DNA). StrandBrite™ Green Fluorimetric RNA Quantitation Kit includes StrandBrite™ RNA Green with an optimized and robust protocol. It provides a convenient and sensitive method for quantifying RNA in solutions.

Kit Components

Components	Amount
Component A: StrandBrite™ RNA Green	50 µL (200X in DMSO)
Component B: 10X Assay Buffer	5 mL
Component C: Ribosomal RNA Standard	20 µL (2 mg/mL)

Sample Protocol for One 96-well Plate

The following protocol is an example for quantifying RNA with StrandBrite™ Green Fluorimetric RNA Quantitation Kit**.** Allow all the components to warm to room temperature before opening. To 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 addressing the mutagenicity or toxicity of StrandBrite™ RNA Green. 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 particular caution.

1. Preparing 1X assay buffer

Prepare a 1X assay buffer by diluting the 10X assay buffer (Component B) with sterile, distilled, nuclease-free water.

2. Preparing StrandBrite™ RNA Green working solution

Prepare StrandBrite™ RNA Green working solution by making a 200-fold dilution of the concentrated DMSO solution in 1X assay buffer (from Step 1). For example, add 10 µL of StrandBrite™ RNA Green (Component A) into 2 mL of 1X assay buffer. 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 20 µg/mL):

3.1 Add 10 µL of 2 mg/mL RNA stock solution (Component C) to 990 µL of 1X assay buffer (from Step 1) to have 20 µg/mL RNA solution, and then perform 1:2 serial dilutions to get approximately 20, 10, 5, 2.5, 1.25, 0.625, 0.313, and 0 µg/mL.
Note: Unused Ribosomal RNA Standard (Component C) should be divided into single use aliquots in nuclease-free plastic vials and stored at ≤ -20 ºC.

3.2 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*

Table 2. Reagent composition for each well

RNA Standard	Blank Control	Test Sample
Serial dilutions* 100 µL	1X assay buffer 100 µL	100 µL

**Note: Add the serially dilutions of RNA standards from 0.3 to 20 µg/mL into wells from RS1 to RS7 in duplicate.*

4. Run RNA assay:

4.1 Add 100 µL of StrandBrite™ RNA 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™ RNA 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 fluorescence microplate reader at Ex/Em = 490/540 nm (cutoff at 515 nm).
Note: To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

Data Analysis

The fluorescence reading in blank well (with 1X assay buffer only) is used as a control, and is subtracted from the values of those wells 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.

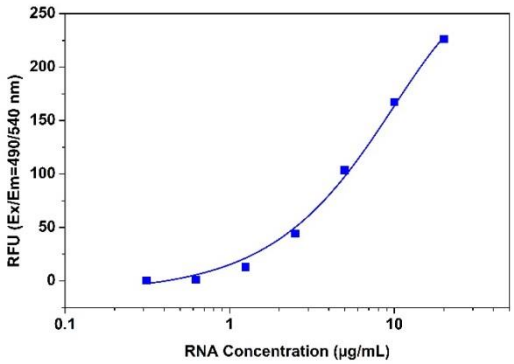

Figure 1. RNA dose response measured with StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat#17657) in a solid black 96-well microplate using a Gemini microplate reader (Molecular Devices).

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.