StrandBrite™ Green Fluorimetric RNA Quantitation Kit *High Selectivity*

Catalog number: 17657
Unit size: 100 Tests

Component	Storage	Amount
Component A: StrandBrite™ RNA Green	Freeze (< -15 °C)	1 vial (50 µL, 200X in DMSO)
Component B: 10X Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (5 mL)
Component C: RNA Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (20 µL, 2 mg/mL)

OVERVIEW

The major challenge to analyze RNA in live cells is the interferences caused by DNA. To address these difficulties, AAT Bioquest has developed the StrandBrite™ RNA Green, an excellent RNA-selective probe that generates significantly enhanced green fluorescence upon binding to RNA. It has been successfully used for flow cytometric analysis of live cells. StrandBrite™ RNA Green readily gets into live cells. It has the excitation/emission of 490/540 nm. In the DNase digest test, no significant change of fluorescence intensity in fixed cells stained with StrandBrite RNA Green was observed. In contrast, after RNase digestion, the initial fluorescence signal decreased immediately. These results indicate that initial fluorescence signal was generated from the specific interaction of StrandBrite RNA Green with RNA in cells. Short exposure of live cells to antinomycin D did cause inhibition of RNA synthesis during 6 hours after drug removal in a dose-dependent manner. These data demonstrate that StrandBrite RNA Green is a sensitive RNA-selective dye for staining nucleolar RNA in live and fixed cells. StrandBrite RNA Green has less DNA interferences than the commonly used SYTO® RNASelect™ dye. StrandBrite™ RNA Green is a highly RNA-selective fluorescent probe. Due to its excellent cell permeability and spectral properties, it has been successfully used for flow cytometric RNA analysis and fluorescence microscope in live cells. It can be well excited with the 488 nm blue laser and monitored in FITC channel. StrandBrite™ RNA Green provides a valuable method for identifying and labeling cells with a single incubation step and can discriminate RNA from DNA with better selectivity than the commonly used SYTO® RNASelect™.

AT A GLANCE

Protocol Summary

1. Prepare StrandBrite™ RNA Green working solution (100 µL)
2. Add RNA standards or test samples (100 µL)
3. Incubate at room temperature for 2 - 5 minutes
4. Monitor the fluorescence intensity at Ex/Em = 490/540 nm (Cutoff = 515 nm)

Important 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 nucleasefree water, and sterile, disposable polypropylene plastic ware for reagent preparation. 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.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	540 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 (1X)
Dilute 10X Assay Buffer (Component B) in sterile, distilled, nuclease-free water to make 1X Assay Buffer.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17657

RNA standard
Add 10 µL of 2 mg/mL RNA Standard (Component C) to 990 µL of 1X Assay buffer to make 20 µg/mL RNA standard solution (RS7). Take 20 µg/mL RNA standard solution (RS7) and perform 1:2 serial dilutions in 1X Assay buffer to get serially diluted RNA standards (RS6 - RS1).

PREPARATION OF WORKING SOLUTION

Add 10 µL of StrandBrite™ RNA Green (Component A) into 1.99 mL of 1X Assay buffer to make StrandBrite™ RNA Green working solution. Protect StrandBrite™ RNA Green 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, this solution should be used 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. RS=RNA Standards (RS1 - RS7, 0.313 to 20 µg/mL); 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.

Well	Volume	Reagent
RS1 - RS7	100 µL	Serial Dilutions (0.313 to 20 µg/mL)
BL	100 µL	1X Assay Buffer
TS	100 µL	test sample

1. Prepare RNA standards (RS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 100 µL of StrandBrite™ RNA Green working solution to each well of 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 StrandBrite™ RNA Green 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, protected from light.

4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 490/540 nm (Cutoff = 515 nm).

   Note To minimize photobleaching effects, keep the time for fluorescence measurement constant for 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 Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

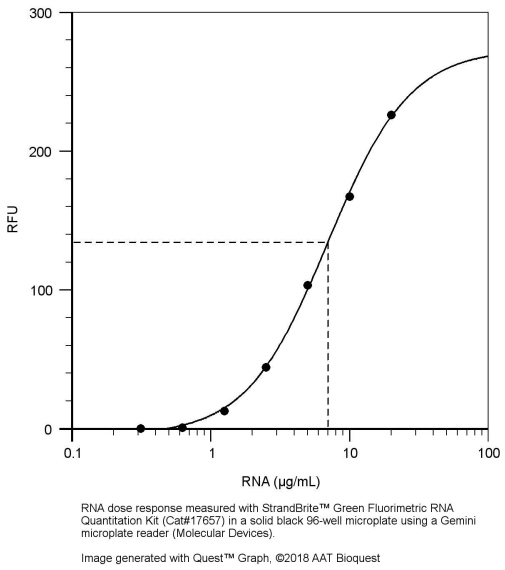

Figure 1. RNA dose response measured with StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat#17657) in a solid black 96-well microplate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER

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