StrandBrite™ Green Fluorimetric RNA Quantitation Kit

Catalog number: 17656
Unit size: 100 Tests

Component	Storage	Amount
Component A: StrandBrite™ Green	Freeze (< -15 °C), Minimize light exposure	1 vial (0.5 mL, 200X in DMSO)
Component B: 10X Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: RNA Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (0.2 mL, 100 µg/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. 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.

AT A GLANCE

Protocol Summary

1. Add 1 mL RNA standards or test samples in each cuvette
2. Add 1 mL StrandBrite Green™ working solution
3. Incubate at RT for 2-5 minutes
4. Monitor the fluorescence at Ex/Em=490/545 nm

Important 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. 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 particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

KEY PARAMETERS

Spectrofluorometer

Excitation	490 nm
Emission	545 nm
Cutoff	515 nm

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 stock solution (1X)
Prepare a 1X Assay Buffer stock solution by diluting the concentrated 10X Assay Buffer (Component B) with sterile, distilled, nuclease-free water.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17656

RNA standard
Add 20 µL of 100 µg/mL RNA stock solution (Component C) to 1.98 mL of 1X Assay Buffer to have 1 µg/mL RNA solution, and then perform 1:3 serial dilutions to get remaining serially diluted RNA standards. Note: Unused Ribosomal RNA Standard (Component C) should be divided into single use aliquots in nuclease-free plastic vials and stored at -20°C.

PREPARATION OF WORKING SOLUTION

Prepare StrandBrite™ Green working solution by making a 200-fold dilution of the concentrated DMSO solution in 1X assay buffer. For example, to prepare enough working solution to assay 10 samples in a 2 mL final volume, add 50 µL of StrandBrite™ Green (Component A) into 10 mL of 1X assay buffer. 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. For best results, this solution should be used within a few hours of its preparation.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 1 mL of StrandBrite™ Green working solution to each cuvette containing 1 mL of the RNA standard, blank control, and test samples to make the total RNA assay volume of 2 mL/cuvette.

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

3. Monitor the fluorescence increase with a spectrofluorometer at Ex/Em = 490/545 nm.

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

EXAMPLE DATA ANALYSIS AND FIGURES

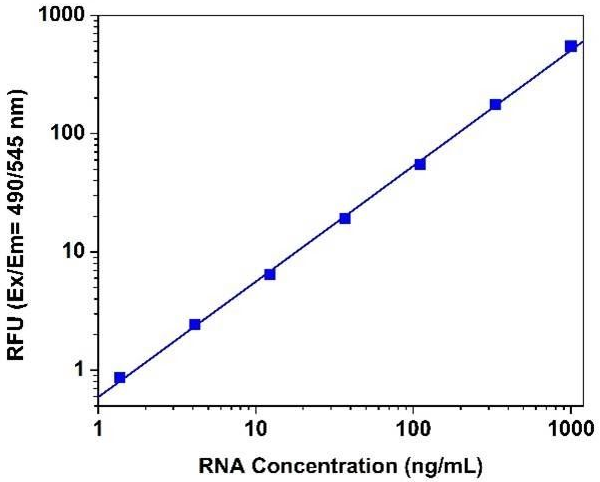

Figure 1. RNA dose response with StrandBrite™ Green in cuvettes and measured using a Cary eclipse spectrofluorometer.

DISCLAIMER

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