Portelite™ Fluorimetric RNA Quantitation Kit*Optimized for Cytocite™ and Qubit™ Fluorometers*

Catalog number: 17658, 17659
Unit size: 100 Tests, 500 Tests

Component	Storage	Amount (Cat No. 17658)	Amount (Cat No. 17659)
Component A: StrandBrite™ Green	Freeze (< -15 °C), Minimize light exposure	0.25 mL (200X in DMSO)	1.25 mL (200X in DMSO)
Component B: Assay Buffer	Refrigerated (2-8 °C)	1 bottle (50 mL)	250 mL
Component C: RNA Standard #1	Refrigerated (2-8 °C)	1 mL (RNA: 0 ng/µL)	5 mL (RNA: 0 ng/µL)
Component D: RNA Standard #2	Freeze (< -15 °C)	4 X 250 µL (RNA: 10 ng/µL)	10 X 500 µL (RNA: 10 ng/µL)

OVERVIEW

Detecting and quantitating small amounts of RNA is extremely important for a wide variety of biological applications 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 absorbance-based method is limited by the interferences caused from proteins, free nucleotides and other UV absorbing compounds. The use of sensitive and selective 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 CytoCite™ or Qubit™ 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. Prepare StrandBrite™ RNA working solution
2. Add 190 µL StrandBrite™ RNA working solution into each 0.2 mL PCR tube
3. Add 10 µL RNA Standards or test samples into each tube
4. Incubate at room temperature for 2 minutes
5. Monitor fluorescence with CytoCite™ or Qubit™ fluorometer

Important Note

Bring all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Qubit Fluorometer

Emission	530 nm
Excitation	480 nm
Instrument specification(s)	0.2 mL PCR vial

CytoCite Fluorometer

Emission	530 nm
Excitation	480 nm
Instrument specification(s)	0.2 mL PCR vial

PREPARATION OF WORKING SOLUTION

StrandBrite™ RNA working solution

Make a 200-fold dilution of StrandBrite™ Green (Component A) in Assay Buffer (Component B). For example, to prepare enough working solution for 8 samples, add 5 µL of StrandBrite™ Green (Component A) into 1 mL of Assay Buffer (Component B).

Note: Protect the working solution from light by covering it with foil or placing it in the dark. 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

The acceptable sample volume could be a range from 1~20 µL depending on the estimate concentration of RNA sample. The recommend sample volume is 10 µL with the RNA concentration in 0.01~10 ng/µL range. If other sample volume is being used, please adjust the dilution factor in the concentration calculations.

The following protocol is generated based on10 µL sample volume with the RNA concentration in 0.01~10 ng/µL range.

1. Add 190 µL StrandBrite™ Green working solution into each CytoCite™ sample tube (#CCT100) or equivalent 0.2 mL PCR tube.

   Note: Use thin-wall, polypropylene, clear 0.2 mL PCR tubes such as#CCT100.

2. Add 10 µL RNA standard #1 and #2 or test samples into each tube, and then mix by vortexing 2~3 seconds.
3. Incubate all tubes at room temperature for 2 minutes.
4. Insert the samples into CytoCite™ or Quibit™ and monitor the fluorescence with green fluorescence channel. Follow the procedure appropriate for CytoCite™ Fluorometer. For instructions, see: https://devices.aatbio.com/documentation/user-manual-for-cytocite-fluorometer

PREPARATION OF STANDARD CALIBRATION CURVE (Optional)

For StrandBrite™ assays, you have the choice to make a calibration curve with the RNA standards. Here is a brief protocol to generate a customized RNA standard curve:

1. Perform 1/3 serial dilution with Assay Buffer to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 ng/µL RNA standard dilutions using RNA Standard #2.
2. Add 190 µL StrandBrite™ Green working solution into each tube.
3. Add 10 µL RNA standards or test samples into each tube, and then mix by vortexing 2~3 seconds.
4. Incubate the reaction at room temperature for 2 minutes.
5. Insert the samples into CytoCite™ and monitor the fluorescence with green fluorescence channel.

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 Concentration samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

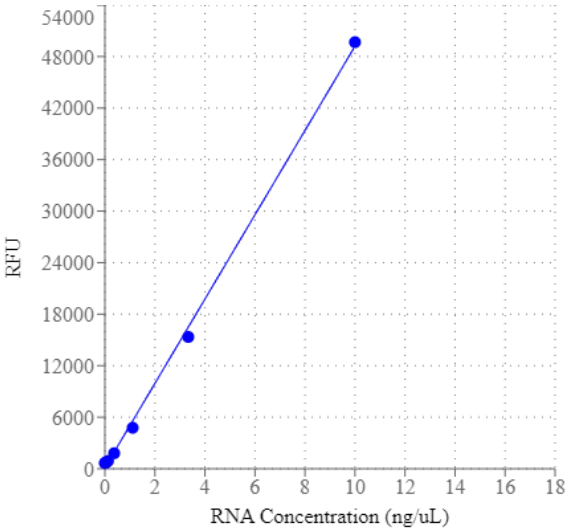

Figure 1. RNA standard curve generated using StrandBrite™ Green Fluorimetric RNA Quantitation Kit. Fluorescence intensity was quantified using green fluorescence channel; regression model was calculated using linear fit.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.