A Modulus™ Method for RNA Quantitation Using

Quant-iT™ RNA

1. INTRODUCTION

The Modulus™ Fluorometer from Turner BioSystems in combination with the Quant-iT™ RNA Assay kit from Molecular Probes provides an accurate method for quantitation of RNA in small volumes (100 µL). The Quant-iT RNA assay is highly selective for RNA over double-stranded DNA. When analyzed in combination with the Modulus Fluorometer and the Red Fluorescence Optical Kit, the signal is linear from 0.8-50 ng RNA in 100 µL final volume (Figure 1). The Quant-iT RNA Assay tolerates salts, solvents, detergents, protein and other common contaminants.

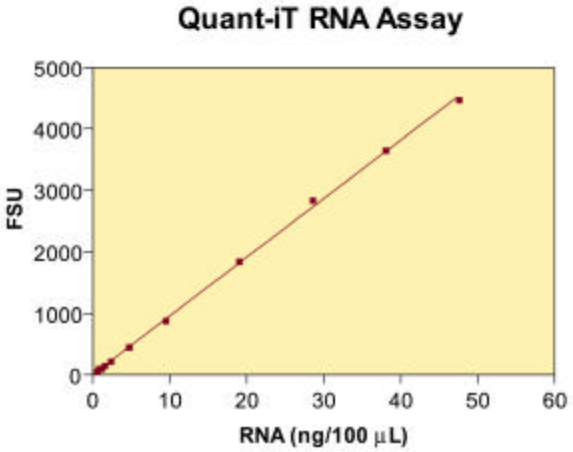

Figure 1. *rRNA and Quant-iT RNA analyzed using the Modulus and the Red Fluorescence Optical Kit. 5 µL of each standard was added to 100 µL of Quant-iT RNA reagent. 100 µL of this mixture was susequently transferred to a minicell cuvette and Modulus measured under the Raw Fluorescence Mode of each dilution.*

2. MATERIALS REQUIRED

- Modulus Fluorometer (P/N 9200-000 or 9200-002)
- Red Fluorescence Optical Kit (P/N 9200-043)
- Quant-iT RNA Assay Kit (Molecular Probes, Q33140)
- Minicell Cuvettes (P/N 7000-950) and Minicell Adaptor (P/N 9200-928)

3. EXPERIMENTAL PROTOCOL

3.1 Reagent Preparation

NOTE: Handling, storage and use of the reagent should be performed in accordance with the product information sheet supplied by Molecular Probes, Inc.

The Quant-iT RNA Reagent is supplied as a 1 mL concentrated dye solution in anhydrous dimethylsulfoxide (DMSO). On the day of the experiment, equilibrate kit contents to room temperature. Prepare a working solution of the Quant-iT RNA Reagent by making a 1:200 dilution of the concentrated dye solution in Quant-iT RNA buffer. Prepare this solution in a plastic container as the reagent may absorb to glass surfaces. Protect the working solution from light by covering it with foil or placing it in the dark.

NOTE: For best results, use this solution within 3 hours of its preparation.

3.2 Instrument Set-Up

3.2.1 Power OFF the Modulus. Insert the Red Fluorescence Optical Kit and Minicell Adaptor according to the *Operating Manual*.

3.2.2 Turn ON the Modulus. Allow a 5-minute warm up period before calibration.

3.3 Calibration

3.3.1 Add 5 µL of each standard to a microcentrifuge tube containing 100 µL of the Quant-iT RNA reagent working solution. Mix by inversion.

3.3.2 Transfer 105 µL of each standard to a minicell cuvette.

3.3.3 Calibrate the Modulus with as many as 5 of the standards. Choose “ng/µL” for the unit of measure. Use the 0 ng/µL standard for the blank solution. To optimize performance and accuracy, choose the 5 standards that are closest in range to a

typical sample. Enter the standards in order of increasing concentration.

3.3.4 Save the calibration for future use (optional).

3.4 Sample Analysis

3.4.1 Add 5 µL of each sample to a microcentrifuge tube containing 100 µL of the Quant-iT RNA Reagent working solution. Mix by inversion.

3.4.2 Transfer 105 µL of each sample to a minicell cuvette.

3.4.3 Read each sample. The concentration of the sample in ng/µL appears on the touchscreen.

NOTE: It is not necessary to run a standard curve after calibration. To check the linearity of the calibration, re-read the standards as samples.

4. PATENTS AND TRADEMARKS

Quant-iT is a registered trademark of Molecular Probes,Inc.

Modulus is a trademark of Turner BioSystems, Inc.

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