

A Modulus™ Single Tube Fluorometer Method for Quant-iT™ Protein



1. INTRODUCTION

The Modulus™ Single Tube Fluorometer from Turner BioSystems in combination with the Quant-iT™ Protein Assay kit from Molecular Probes provides an accurate method for quantitation of protein in small volumes (100 μ L). The Quant-iT Protein assay is highly selective for protein and tolerates common contaminants including salts, solvents or DNA, but not detergents.

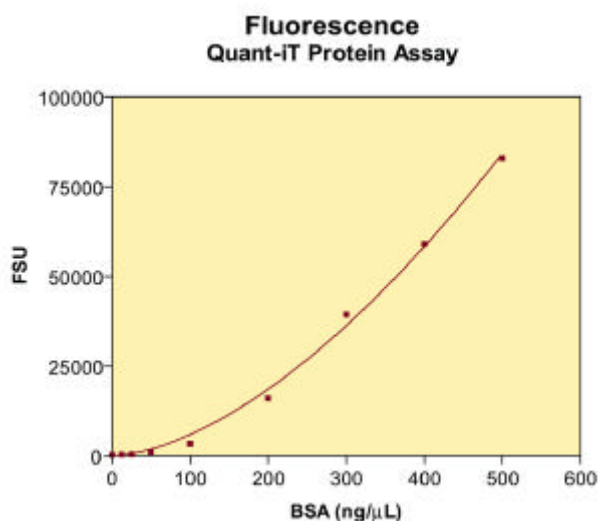


Figure 1. BSA and Quant-iT Protein analyzed using the Modulus and the Blue Fluorescence Optical Kit. 5 μ L of each standard was added to 100 μ L of Quant-iT RNA reagent. 100 μ L of this mixture was transferred to a minicell cuvette. The Modulus measured raw fluorescence of each dilution.

2. MATERIALS REQUIRED

- ❖ Modulus™ Single Tube Fluorometer (P/N 9200-000 or 9200-002)
- ❖ Blue Fluorescence Optical Kit (P/N 9200-040)
- ❖ Quant-iT Protein Assay Kit (Molecular Probes, Q33210)
- ❖ 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 Protein Reagent is supplied as a 1 mL concentrated dye solution in 1,2-propanediol. On the day of the experiment, equilibrate kit contents to room temperature. Prepare a working solution of the Quant-iT Protein Reagent by making a 1:200 dilution of the concentrated dye solution in Quant-iT Protein buffer. Prepare this solution in a plastic container as the reagent may adsorb 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 Blue Fluorescence Optical Kit and Minicell Adaptor according to *Operating Manual*.
- 3.2.2 Turn ON the Modulus. Allow a 5-minute warm up period.

3.3 Standard Curve

- 3.3.1** Add 5 μ L of each standard to a microcentrifuge tube containing 100 μ L of the Quant-iT Protein Reagent working solution. Mix by inversion.
- 3.3.2** Transfer 105 μ L of each standard to a minicell cuvette.
- 3.3.3** The default mode for the Modulus is the Raw Fluorescence Mode. Read all standards in the Raw Fluorescence Mode and record the readings.
- 3.3.4** Plot amount of the standard vs. fluorescence (FSU) and fit a curve through the data points.

3.4 Sample Analysis

- 3.4.1** Add 5 μ L of each sample to a microcentrifuge tube containing 100 μ L of the Quant-iT RNA reagent working solution. Mix by inversion.
- 3.4.2** Transfer 105 μ L of each sample to a minicell cuvette.
- 3.4.3** Read each sample in the Raw Fluorescence Mode.
- 3.4.4** Use the standard curve plot from step 3.3.4 to determine the amount of protein in the samples.

4. PATENTS AND TRADEMARKS

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

Modulus is a trademark of Turner BioSystems, Inc.

5. ABOUT MOLECULAR PROBES, INC.

Orders for Molecular Probes' products may be placed by:

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6. ABOUT TURNER BIOSYSTEMS, INC.

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