A Modulus™ Microplate Fluorometer Method for Quant-iT™ Protein Assay Kit

1. INTRODUCTION

Use of the Modulus™ Microplate Fluorometer from Turner BioSystems, Inc. in combination with the Quant-iT™ Protein Assay Kit from Invitrogen provides an accurate method for quantifying protein. Quant-iT™ Protein Assay is highly selective for protein and tolerates common contaminants including salts, solvents, or DNA, but not detergents. The assay is performed at room temperature and the signal is stable for 3 hours.

Protein level determination has never been easier than through use of the Modulus™ Microplate Fluorometer. Simply add standards and unknowns to a microplate, add a working solution of Quant-iT™ Protein Reagent, then mix and read. Levels of protein from 0.125 - 7 µg can be assayed.

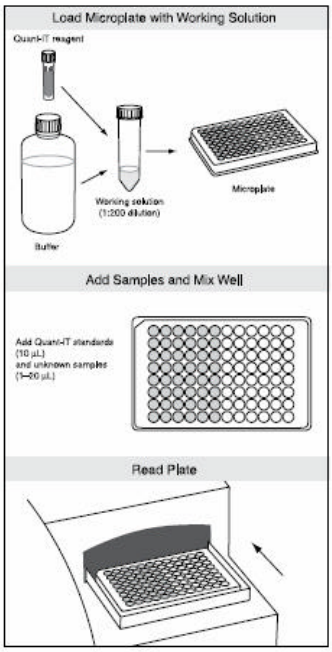


Figure 1. Quant-iT™ Protein Assay read on a microplate reader.

2. MATERIALS REQUIRED

- Modulus™ Microplate Multimode Reader
- Fluorescence Optical Kit – Blue, 475/515 - 580 nm
- Black 96-well microplates, FluoTrac 200 (E&K Scientific, EK-25076)
- Quant-iT™ Protein Assay Kit (Invitrogen, Q33210) containing:
    - Quant-iT™ Protein Reagent (Component A), 1.0 mL
    - BSA standards, set of 8 at500 uL each
    - Quant-iT™ Protein Buffer (Component B), 250 mL

Note: Handling, storage, and use of reagents should be performed in accordance with the product information sheet provided by Molecular Probes, Inc.

3. EXPERIMENTAL PROTOCOL

3.1 Reagent Preparation

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 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 reagent may adsorb to glass surfaces. Protect the working solution from light by covering it with foil or placing it in a dark environment.

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

3.2 Standard Curve and Sample Analysis

1. Add 200 µL of working solution to each microplate well.

2. Load 10 µL of each BSA standard (Component C) into separate microplate wells and mix adequately.

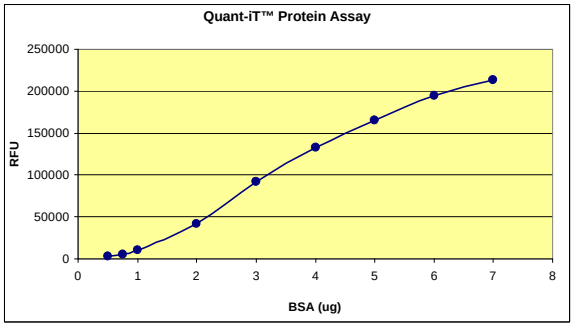
Note: It is recommended to obtain duplicates or triplicates of each standard for best results in determining the accuracy of the standard curve.

3. Load 1 - 20 µL of each unknown sample into separate microplate wells and mix adequately.

4. Set up the Modulus™ Microplate Fluorometer as per instructions in the *Operating Manual* and read the assay plate.

5. Plot the amount of standard versus fluorescence (RFU) and fit a curve through the data points.

6. Use the standard curve plot to determine the amount of protein in the unknown samples.



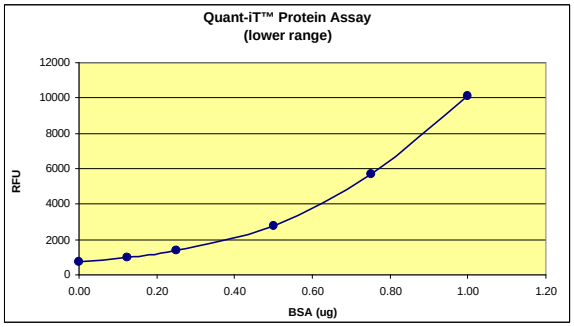


Figure 2. Protein sensitivity of the Quant-iT™ protein assay. The provided BSA standards were assayed in triplicate using 10 µL of BSA. The response curve is pseudo-linear from 0.5 - 6 µg and a straight line may be fit to this range of data points. The %CV for all standards was less than 5%.

4. RESULTS

Sensitivity:

- Typically < 0.6 µg/mL of BSA

Instrument Dynamic Range:

- Up to 6 orders of magnitude within the dynamic range

Minimum Detection Limit:

- 58 ng/mL of BSA
- Calculated using 3 x standard deviation of the assay background, n = 24

5. CONCLUSION

The Quant-iT™ Protein Assay Kit makes for an easy, reliable, and accurate method to quantify protein samples when used in conjunction with the Modulus™ Microplate Fluorometer from Turner BioSystems, Inc. The superior sensitivity and performance of the Modulus™ Microplate Fluorometer allows for protein detection down to the 125-ng range.

The Modulus™ Microplate Fluorometer achieves superior performance by use of a dedicated fluorescence detector. The detector is not shared with any other detection modes. The individual Fluorescent Optical Kit of the Modulus™ Microplate Fluorometer uses solid-state optics and a powerful wavelength-matched LED to deliver excellent sensitivity and dynamic range.

The modular approach of the Modulus™ Microplate Fluorometer allows for instrument capability expansion as needs in the lab change. Luminescence and/or Absorbance Detection Modules as well as other accessories can be added after initial purchase.

Superior performance, ease of use, and utmost flexibility makes the Modulus™ Microplate Reader an ideal choice for today's life science laboratory.

6. PRECAUTIONS

Care should be taken when handling the Quant-iT™ protein reagent. This reagent is known to bind to nucleic acids and is provided in solution form with 1,2-propanediol. Treat the reagent with the same safety precautions as all other potential mutagens and dispose of dye in accordance with local regulations.

TRADEMARKS

ABOUT TURNER BIOSYSTEMS, INC.

Turner BioSystems, Inc. manufactures more fluorometers and research-grade luminometers than any other company in the world.

Orders for Turner BioSystems products may be placed by contacting our Sales Department at:

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