Portelite™ Fluorimetric Protein Quantitation Kit

Catalog number: 11110
Unit size: 200 Tests

Component	Storage	Amount
Component A: Prolite™ Orange (200X)	Room temperature	1 vial (300 µL)
Component B: BSA Standard 1 (400 µg/mL)	Refrigerate (2-4 °C)	1 vial (1.5 mL)
Component C: BSA Standard 2 (200 µg/mL)	Refrigerate (2-4 °C)	1 vial (1.5 mL)
Component D: BSA Standard 3 (0 µg/mL)	Refrigerate (2-4 °C)	1 vial (1.5 mL)
Component E: Sample Dilution Buffer	Room temperature	1 bottle (100 mL)

OVERVIEW

Protein quantification is necessary in protein purification, electrophoresis, cell biology, molecular biology and other research applications. Biuret, Lowry, BCA and Bradford assays are routinely used for estimating protein concentration. However, these colorimetric assays are less sensitive, and require large sample volume to ensure higher accuracy. Our Portelite™ Fluorimetric Protein Quantitation Kit is significantly more sensitive than existing standard colorimetric measurements, e.g., Bradford and Bicinchoninic acid (BCA) assays. Prolite™ Orange used in the kit is intrinsically non-fluorescent in aqueous solution, but reacts rapidly with proteins and generates bright fluorescence. Portelite™ Fluorimetric Protein Quantitation Kit provides a rapid method for quantifying protein concentration in solutions. As little as 50 ng/mL of BSA can be detected. The kit can be performed with the Qubit® Fluorometer. It can be completed within 15 minutes with the fluorescence signal easily monitored at Ex/Em = 485/590 nm.

AT A GLANCE

Protocol summary

1. Prepare and add BSA standards or test samples (10 µL)
2. Prepare and add Prolite™ Orange working solution (190 µL)
3. Incubate at room temperature for 15 minutes
4. Monitor fluoroscence intensity at Ex/Em = 485/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	485 nm
Emission:	590 nm
Cutoff:	560 nm
Recommended plate:	Solid black

PREPARATION OF WORKING SOLUTION

Prolite™ Orange working solution:
Add 1 µL of Prolite™ Orange (200X) (Component A) to 199 µL of Sample Dilution Buffer (Component E) and mix them well.

Note Do not mix the working solution in a glass container.

SAMPLE EXPERIMENTAL PROTOCOL

This protocol is generated based upon Qubit® Fluorometer.

Run protein assay

1. Add 190 µL/well of Prolite™ Orange working solution into each tube.

   Note Use thin-wall, polypropylene, clear 0.5 mL PCR tubes such as Invitrogen™ Qubit® Assay Tubes (Cat# Q32856) or Axygen PCR-05-C tubes (VWR, Cat# 10011-830). Other types of tubes can have auto fluorescence and may interfere with the assay.

2. Add 10 µL BSA standards (Component B, C, D) or 10 µL samples into the 190 µL Prolite™ Orange working solution tube to make the final assay volume 200 µL/tube.

3. Incubate the reaction at room temperature for 15 minutes.

   Note Protect the samples from light and avoid holding the samples in hands.

4. Insert the samples into Qubit® and monitor the fluorescence at Ex/Em = 485/590 nm.

Brief protocol for Qubit® fluorometer

1. Press Protein on the Home screen of the Qubit® Home screen and proceed to press Read standards.
2. Insert each of the 3 tubes contains standards into the sample chamber.
3. Close the lid and press Read standards.
4. The instrument displays the results and generates calibration curve.
5. Press Run samples and select sample volume to 10 µL.
6. Insert the sample tube into the sample chamber.
7. Close the lid and press Read tube.
8. The instrument displays the results on the assay screen. The top value is the original sample concentration and bottom value is the diluted concentration.

EXAMPLE DATA ANALYSIS AND FIGURES

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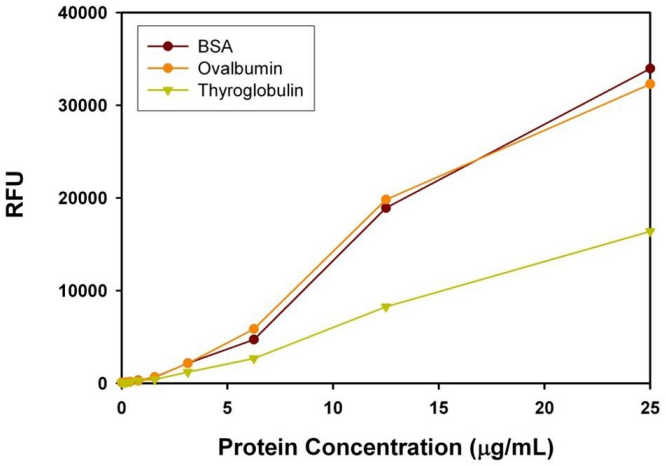

Figure 1.

Serial dilutions of BSA, chicken-egg ovalbumin, porcine thyroglobulin were measured at Ex/Em 485/590 nm using Portelite™ Fluorimetric Protein Quantitation Kit with Qubit® Fluorometer. As low as 50 ng/mL of protein can be detected.

DISCLAIMER