Portelite™ Fluorimetric Protein Quantitation Kit *Optimized for CytoCite™ and Qubit™ Fluorometers*

Catalog number: 11109, 11111
Unit size: 100 Tests, 500 Tests

Component	Storage	Amount	
		Cat No. 11109	Cat No. 11111
Component A: Prolite™ Orange (200X)	Room temperature	1 vial (300 µL)	1 vial (1.5 mL)
Component B: BSA Standard 1 (0 ng/uL)	Refrigerate (2-8 °C)	1 vial (1 mL)	1 bottle (5 mL)
Component C: BSA Standard 2 (200 ng/uL)	Refrigerate (2-8 °C)	1 vial (1 mL)	1 bottle (5 mL)
Component D: BSA Standard 3 (400 ng/uL)	Refrigerate (2-8 °C)	1 vial (1 mL)	1 bottle (5 mL)
Component E: Sample Dilution Buffer	Room temperature	1 bottle (60 mL)	300 mL (3 bottles-100 mL each)

OVERVIEW

Protein quantification is an essential task 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 accuracy. Our Portelite™ Fluorimetric Protein Quantitation Kit is significantly more sensitive than existing colorimetric protein measurements, e.g., Bradford and Bicinchoninic acid (BCA) assays. Prolite™ Orange used in the kit is non-fluorescent in aqueous solution, but reacts rapidly with proteins and generates bright fluorescence. The Portelite™ Fluorimetric Protein Quantitation Kit provides a simple method for quantifying protein concentration in solutions. The assay has dynamic range from 12.5 ug/mL to 5 mg/mL of BSA. The kit is optimized for Cytocite™ and Qubit™ fluorometers. It can be used for (1) studying protein/protein interactions; (2) measuring column fractions after affinity chromatography; (3) estimating recovery of membrane proteins from cell extract; and (4) high-throughput screening of fusion proteins.

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) in a 0.2 mL PCR tube (Cat# CCT100)
3. Incubate at room temperature for 15 minutes
4. Monitor fluoroscence with CytoCite™ or Qubit fluorometer

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

KEY PARAMETERS

Instrument:	CytoCite Fluorometer
Excitation:	480 nm
Emission:	510-580 nm
Instrument specification(s):	0.2 mL, thin-wall PCR tube

Instrument:	Qubit Fluorometer
Excitation:	480 nm
Emission:	510-580 nm
Instrument specification(s):	0.2 mL, thin-wall PCR tube

PREPARATION OF WORKING SOLUTION

Prolite™ Orange working solution:
Add 5 µL of Prolite™ Orange (200X) (Component A) to 995 µL of Sample Dilution Buffer (Component E) and mix them well.

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

Note Prepare the amount as needed.

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.
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 CytoCite™ and monitor the fluorescence with Green fluorescent channel. Follow the procedure appropriate for CytoCite™ Fluorometer. See the link below for detailed instructions: https://devices.aatbio.com/documentation/user-manual-for-cytocite-fluorometer

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.

PREPARATION OF STANDARD SOLUTION

For CytoCite™ Fluorometer assays, you have the choice to run a calibration with your own protein standards. Here is a brief protocol to generate a customized

protein standard curve.

1. Prepare a protein solution of 400µg/ml (400 ng/µL) in PBS buffer.
2. Perform 1:2 serial dilution with PBS buffer to get 200, 100, 50, 25, 12.5 ng/µl serial standard dilutions.
3. Add 190 µL of Prolite™ Orange working solution into a 0.2 mL PCR tube.
4. Add 10 µL standards or 10 µL samples into each tube.
5. Incubate the reaction at room temperature for 15 minutes.
6. Insert the samples into CytoCite™ and monitor the fluorescence with Green fluorescent channel.

EXAMPLE DATA ANALYSIS AND FIGURES

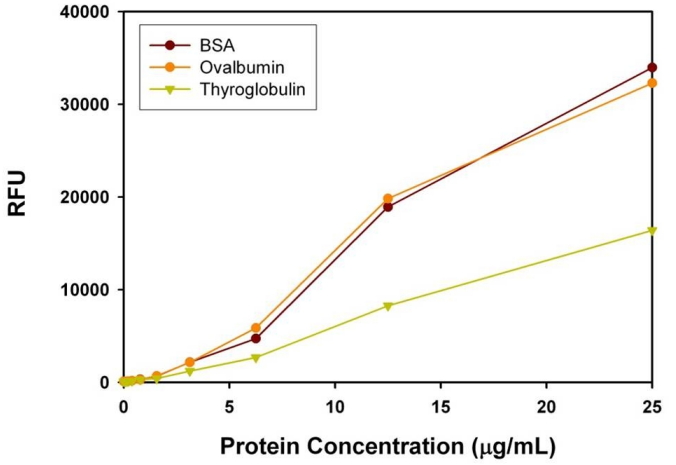

Figure 1.

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

DISCLAIMER