Amplite™ Fluorimetric Protein Quantitation Kit *Orange Fluorescence*

Catalog number: 11105
Unit size: 500 tests

Component	Storage	Amount
Component A: 500X Prolite™ Orange	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL)
Component B: BSA Standard (1 mg/mL)	Freeze (<-15 °C), Minimize light exposure	1 vial (0.5 mL)
Component C: Protein Enhancer	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component E: Protein Enhancer Buffer	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)

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 Amplite™ 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 Amplite™ Fluorimetric Protein Quantitation Kit provides a simple method for quantifying protein concentration in solutions. As little as 0.1 µg/mL of BSA can be detected. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format. It can be completed within 30 minutes with the fluorescence signal easily monitored. This kit has been 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 Prolite™ Orange working solution (50 µL)
2. Add BSA standards and/or test samples (50 µL)
3. Incubate at room temperature for 30 minutes
4. Read fluorescence intensity at Ex/Em = 485/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	485 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Protein Enhancer stock solution (500X):
Add 100 µL of Protein Enhancer Buffer (Component E) into the vial of Protein Enhancer (Component C).

Note 20 µL of Protein Enhancer stock solution (500X) is enough for make 10 mL working solution. Unused Protein Enhancer stock solution (500X) should be stored in single use aliquots at -20 °C.

PREPARATION OF STANDARD SOLUTION

BSA standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11105

Note Standards and test samples should be prepared in Protein Enhancer working solution.

PREPARATION OF WORKING SOLUTION

1. Protein Enhancer working solution:
Add 20 µL of Protein Enhancer stock solution (500X) into 10 mL of Assay Buffer (Component D) and mix them well.

2. *Prolite™ Orange Working Solution:*
Add 10 µL of Prolite™ Orange (Component A) into 5 mL of Assay Buffer (Component D) and mix them well.

Note 5 mL of Prolite™ Orange working solution is enough for 1 plate. Prepare enough Prolite™ Orange working solution fresh for each experiment.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of BSA standards and test samples in a solid black 96-well microplate. BS = BSA standard (BS1-BS7); BL = blank control; TS = test sample.

BL	BL	TS	TS
BS1	BS1	...	...
BS2	BS2	...	...
BS3	BS3		
BS4	BS4		
BS5	BS5		
BS6	BS6		
BS7	BS7		

Table 2. Reagent composition for each well

BSA Standard	Blank Control	Test Sample
Serial Dilutions: 50 µL	Protein Enhancer working solution: 50 µL	50 µL

Protein assay

1. Add 50 µL of BSA standard, blank control, and test samples (See table 1 and 2) into a solid black 96-well plate.

Note

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For a 384-well plate, add 20 µL of samples.

2. Add 50 µL/well of Prolite™ Orange working solution into BSA standard, blank control, and test samples to make the total assay volume of 100 µL/well.

   Note For a 384-well plate, add 20 µL of Prolite™ Orange working solution into each well.

3. Incubate the reaction for 10 to 30 minutes at 37 ^{o}C, protected from light.

4. Monitor the the fluorescence increase with a fluorescence plate reader at Ex/Em = 485/590 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

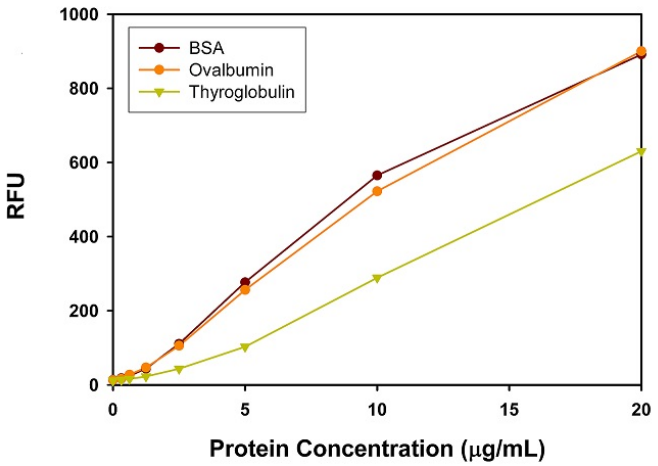

Figure 1.

BSA, chicken-egg ovalbumin, porcine thyroglobulin dose response was measured at Ex/Em 485/590 in a 96-well black plate with the Amplite™ Fluoremetric Protein Quantitation Kit. As low as 0.1 ug/mL of BSA can be detected.

DISCLAIMER