Amplite™ Fluorimetric Fluorescamine Protein Quantitation Kit *Blue Fluorescence*

Catalog number: 11100
Unit size: 200 Tests

Component	Storage	Amount
Component A: Fluorescamine	Freeze (<-15 °C), Minimize light exposure	1 bottle
Component B: DMSO	Freeze (<-15 °C)	1 bottle (5 mL)
Component C: BSA Standard (1 mg/mL)	Freeze (<-15 °C), Minimize light exposure	0.5 mL

OVERVIEW

Fluorescamine is intrinsically non-fluorescent but reacts rapidly with primary aliphatic amines, including those in peptides and proteins, to yield a blue-green-fluorescent derivative. The Amplite™ fluorescamine protein assay kit provides a simple method for quantifying protein concentration in solutions. This Amplite™ fluorescamine protein assay kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. The assay can be completed within 30 minutes. With the Amplite™ fluorescamine protein assay kit, as little as 3 ug/mL of BSA can be detected.

AT A GLANCE

Protocol summary

1. Prepare fluorescamine working solution (25 µL)
2. Add BSA standards or test samples (75 µL)
3. Incubate at room temperature for 5 - 30 minutes
4. Read fluorescence intensity at Ex/Em = 380/470 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	380
Emission:	470
Cutoff:	420
Recommended plate:	Solid black

PREPARATION OF STANDARD SOLUTION

BSA standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11100

Dilute the appropriate amount of BSA Standard 1 mg/mL (Component C) into PBS by performing 1:2 serial dilutions to get serial dilutions of BSA standard (BS7 - BS1).

PREPARATION OF WORKING SOLUTION

Add the whole content of DMSO (Component B) into the bottle of Fluorescamine (Component A), and mix well.

Note 2.5 mL of fluorescamine working solution is enough for 1 plate.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of BSA standards and test samples in a solid black 96-well microplate. BS= BSA Standards (BS1 - BS7, 1.563 to 100 µg/mL), BL=Blank Control, TS=Test Samples.

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.

Well	Volume	Reagent
BS1 - BS7	75 uL	Serial Dilution (1.563 to 100 ug/mL)
BL	75 uL	PBS
TS	75 uL	Test Sample

1. Prepare BSA standards (BS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 30 µL of reagent per well instead of 75 µL.
2. Add 25 µL of fluorescamine working solution to each well of BSA standard, blank control, and test samples to make the total assay volume of 100 µL/well. For a 384-well plate, add 10 µL of fluorescamine working solution into each well instead, for a total volume of 40 µL/well.
3. Incubate the reaction at room temperature for 5 to 30 minutes, protected from light.
4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 380/470 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate BSA samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

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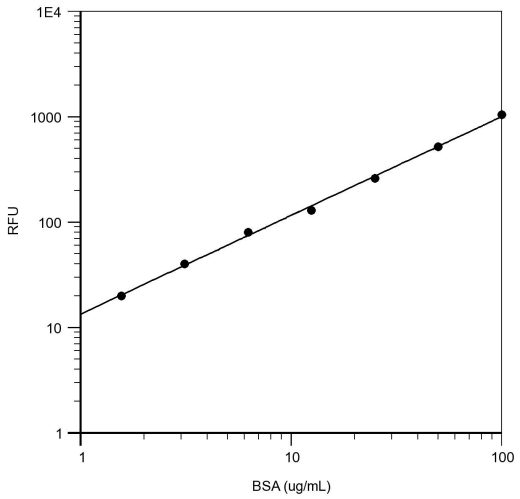

Figure 1. BSA dose response was measured on a solid black 96-well plate with Amplite™ Fluoremetric Fluorescamine Protein Quantitation Assay Kit.

DISCLAIMER