Amplite™ Fluorimetric Fluorescamine Protein Quantitation Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11100 (200 assays)	Keep at 4 °C and avoid exposure to light	Fluorescence microplate readers

Introduction

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 fluorescamine-based protein quantification kit is significantly more sensitive than existing standard colorimetric measurements, e.g., Bradford and Bicinchoninic acid (BCA) assays.

Fluorescamine is intrinsically nonfluorescent but reacts rapidly with primary aliphatic amines, including those in peptides and proteins, to yield a blue-green-fluorescent derivative. The Amplite™ Fluorescamine Protein Quantitation Kit provides a simple method for quantifying protein concentration in solutions. As little as 3 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 at Ex/Em = 380/470 nm. This kit has been used for (1). studying protein/protein interactions; (2). measuring column fractions after affinity chromatography; (3). estimating percent recovery of membrane proteins from cell extract; and (4). high-throughput screening of fusion protein.

Kit Components

Components	**Amount**
Component A: Fluorescamine	1 bottle
Component B: DMSO	1 bottle (5 mL)
Component C: BSA Standard (1 mg/mL)	0.5 mL

Assay Protocol for One 96-Well Plate

Brief Summary

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

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

1. **Prepare Fluorescamine 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. Unused fluorescamine working solution should be stored in single use aliquots at -20 °C and protected from light.
2. **Prepare BSA standard solutions:** Dilute the appropriate amount of BSA Standard, 1 mg/mL(Component C) into PBS to get a BSA concentration of 100-1.56 µg/mL with 2X dilutions.

Table 1 Layout of BSA standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
BS1	CS1	….	….	….	….						
BS2	CS2										
BS3	CS3										
BS4	CS4										
BS5	CS5										
BS6	CS6										
BS7	CS7										

Note: BS= BSA Standards, BL=Blank Control, TS=Test Samples.

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Table 2 Reagent composition for each well

BSA Standard	Blank Control	Test Sample
Serial Dilutions*: 75 µL	PBS: 75 µL	75 µL

**Note: Add the serial dilutions of BSA standard from 100 to 1.56 µg/mL with 2X dilutions into wells from BS1 to BS7 in duplicate (see Step 2).*

3. Run protein assay:

3.1 Add the serial dilutions of BSA standard at 75 µL/well (see Tables 1 & 2) into the 96-well plate. A 0 µg/mL BSA control is included as blank control.

3.2 Add 25 µL/well of fluorescamine working solution (from Step 1) into BSA standard, blank control, and test samples (see Step 2, Table 1) to make the total assay volume of 100 µL/well.
Note: For a 384-well plate, add 30 µL of sample and 10 µL of fluorescamine working solution into each well.

3.3 Incubate the reaction at room temperature for 5 to 30 minutes, protected from light.

3.4 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 380/470 nm.

Data Analysis

The fluorescence in blank wells (with the PBS only) is used as a control, and is subtracted from the values for those wells with protein reactions. A BSA standard curve is shown in Figure 1.The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

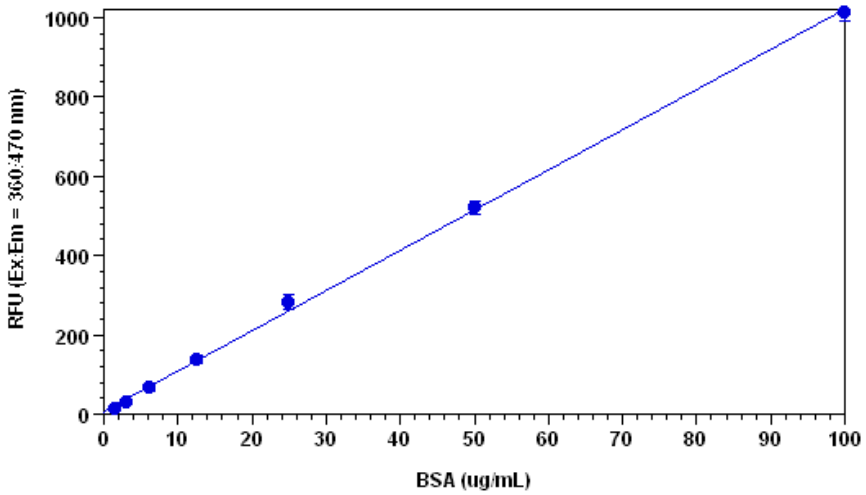

Figure 1. BSA dose response was measured on a 96-well back plate with the Amplite™ Fluoremetric Fluorescamine Protein Quantitation Assay Kit. As low as 0.3 µg/mL of BSA can be detected with 5 minutes incubation time (n=3).

References

1. Eastwood D, Fernandez C, Yoon BY, Sheaff CN, Wai CM. (2006) Fluorescence of aromatic amines and their fluorescamine derivatives for detection of explosive vapors.Appl Spectrosc, 60, 958.
2. Adamou R, Coly A, Douabale SE, Saleck ML, Gaye-Seye MD, Tine A. (2005) Fluorimetric determination of histamine in fish using micellar media and fluorescamine as labelling reagent. J Fluoresc, 15, 679.
3. Eggenreich K, Zach E, Beck H, Wintersteiger R. (2004) Determination of 4-amino-mcresol and 5-amino-o-cresol by high performance liquid chromatography and fluorescence derivatization using fluorescamine. J Biochem Biophys Methods, 61, 35.