

ab324117 – Fluorescamine Protein Assay Kit (Fluorometric)

For quantifying protein concentration in solutions.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324117

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
Fluorescamine	1 bottle	-20°C (Store in the dark)
DMSO	1 bottle (5 mL)	-20°C
BSA Standard (1 mg/mL)	0.5 mL	-20°C (Store in the dark)

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Fluorescence microplate reader capable of measuring absorbance at 380-470 nm
- Solid black plates
- PBS

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.

Preparation of BSA Standard

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

Preparation of Working Solution

1. Add the whole content of DMSO into the bottle of Fluorescamine and mix well.

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

Experimental Protocol

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

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.

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

Table 2. Reagent composition for each well of 96-well microplate.

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.

Data Analysis

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 baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation

For technical support contact information, visit: www.abcam.com/contactus

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