

## Fluorescamine Protein Assay

### Introduction

The Fluorescamine protein dye is a fluorescent stain for quantitating minute amounts of protein and peptides in solution. The Fluorescamine reagent reacts rapidly with primary amines. When activated with the UV LED (365 nm), the fluorescence of the protein-dye complex has an emission wavelength of approximately 470 nm. Used in conjunction with the micro-volume capability of the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, the Fluorescamine reagent provides a highly sensitive means of protein quantitation with minimal consumption of sample. The ability of the NanoDrop 3300 to measure as little as 2 ul of protein samples allows significantly scaled-down reaction volumes, thereby using only a fraction of sample and reagent commonly needed for conventional cuvette-based fluorometers. The Fluorescamine assay has a linear dynamic range of 8—500 ug/ul for BSA.

**\*Notes:** There is considerable protein-to-protein and peptide-to-peptide variation with this assay so use of a purified sample of the particular protein or peptide as a standard is recommended. Buffers containing primary amines are not recommended. Peptides or proteins with blocked primary amines will not give a response with fluorescamine.

### Assay Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2uL pipettor

Materials:

- Low lint laboratory wipes
- Nuclease free sterile amber or foil covered 1.5 mL polypropylene tubes
- Nuclease free sterile clear 1.5 mL polypropylene tubes
- 10 uL sterile nuclease free low retention tips

Reagents:

- Fluorescamine (Acros Organics Cat # 191670250 or Sigma Cat #F9015)
- DMSO Spectroscopy grade
- PBS pH 7.2 (Pierce cat #28372)
- BSA Standard (Pierce cat # 23209) or other protein/peptide standard

### Assay Recommendations

- Mix all solutions gently to avoid micro bubbles.
- Measure 2 ul sample aliquots.
- Remove samples from the optical surfaces by blotting rather than wiping to reduce residual lint fibers from collecting on the sampling pedestals.
- Equilibrate all reagents, unknowns and protein standards to room temperature.
- *Protect the reagent and solutions containing reagent from light*

More information about fluorescamine may be found in the following reference: Udenfriend, S., S. Stein, P. Böhlen, W. Dairman, W. Leimgruber, and M. Weigele (1972) Fluorescamine: A Reagent for Assay of Amino Acids, Peptides, Proteins, and Primary Amines in the Picomole Range, *Science* 178:871-872.

### Protocol (8ug/ml– 500 ug/ml Protein)

1. Prepare a fresh stock of 3 mg/ml Fluorescamine by dissolving 3.0 mg in 1.0 ml of DMSO in an amber tube and mixing thoroughly to dissolve.
2. Prepare 2 fold serial dilutions of your standard in PBS. Mix each stock BSA standard solution and unknown thoroughly and transfer 9 ul of each BSA standard to the respective amber tube.
3. Add 3ul of the stock fluorescamine solution into each assay tube and pipette up and down to mix. The final reaction volume for each standard and unknown should be 12 ul
4. Allow the reaction to incubate at room temperature for 15 minutes. *Note: The assay is stable for a few hours but it is recommended to run the unknown samples to be measured immediately after the standard curve has been established.*
5. Mix well, collect the solution at the bottom of the tube by a brief centrifugation, and proceed to the NanoDrop 3300 standard curve protocol on page 2.

## Standard Curve Protocol

- Clean both sampling pedestals with 2 uL of nuclease free de-ionized water.
- Open upper arm and firmly blot the two pedestals with a dry lab wipe. Make sure there are no traces of lint on the pedestals before continuing.
- Open the operating software. Click on the Protein Quantitation button and select the Fluorescamine method.
- Add 2 uL of assay buffer (no dye, no sample) to the lower pedestal. Lower the arm and click F3 or the Blank button. When the measurement is complete, lift the arm and use a dry laboratory wipe to blot the buffer from both the bottom and upper measurement surfaces. Use a fresh aliquot of buffer to verify a proper baseline.
- Under Measurement type, click on the Standards tab. Highlight the Reference standard.
- Mix the reference solution (assay buffer and dye, no sample) briefly and transfer 2 uL of the solution onto the lower pedestal. Lower the arm and click F1 or the Measure button. A pop up window will ask for confirmation of the units. (Recommended ng/mL or pg/uL)
- Measure up to 5 replicates of the reference solution using a fresh 2 uL aliquot for each measurement.
- Select Standard 1 to enter a value. Enter values for up to 7 standards.
- Mix the standard solution briefly and transfer 2 uL onto the lower pedestal. Lower the arm and click F1 or the Measure button. Measure up to 5 replicates of each standard using a fresh 2 uL aliquot for each measurement.
- Once the standard curve is completed, select the Standard Curve Type (Interpolation, Linear, 2<sup>o</sup> polynomial, 3<sup>o</sup> polynomial) that best fits the standards data set.
- Click on the Sample tab under Measurement Type, and enter the unknown samples' respective ID information. If a dilution of the unknown sample was made, enter the dilution factor in the box below the sample ID window.
- Add 2 ul of the sample and use the F1 key or click the Measure button to initiate the measurement cycle. Use a fresh aliquot of sample for each measurement.

## Performance Data

Typical data for the Fluorescamine protein assay.

Micrograms of BSA per ml	Ave RFU (n=5)	SD	%CV
0	55	6	11
7.8	111	11.5	10
15.6	221	10	4.8
31.3	498	24	4.9
62.5	1226	61	5
125	2313	67	2.9
250	5549	232	4.2
500	10897	188	1.9

Example spectrum Florescamine protein sample.

