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# FluoroRed 600 Protein Assay

(Fluorescence Based Protein Quantification Assay)

(Cat. # 786-1595, 786-1596)



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## INTRODUCTION

FluoroRed 600 Protein Assay is a rapid and highly sensitive fluorescent dye based assay for quantification of proteins in solutions. FluoroRed 600 Protein Assay is comprised of fluorescent amine group binding dye which has different absorption and emission spectrum in conjugated and non-conjugated form. The dye bind to the proteins through their amine groups and upon binding the absorption and emission spectrum shifts to 500/600nm (Ex/Em). The absorption can be read anywhere between  $490\pm 10$  nm and emission between  $600\pm 10$  nm.

The assay is rapid, safe and easy to use when compared with other commercially available fluorescent protein assays. The detection limit of the assay can be as low as 10 ng when performed in microwell assay format with assay volume of 200  $\mu$ l. The linear detection range of the assay between 10 ng to 1  $\mu$ g (Fig.1). The assay is available in 100 and 500 microwell assay format. It can be adapted for measurement with standard fluorometer or minifluorometer by diluting the reaction mixture up to 1 ml using 1 X FluoroRed 600 Reaction Buffer.

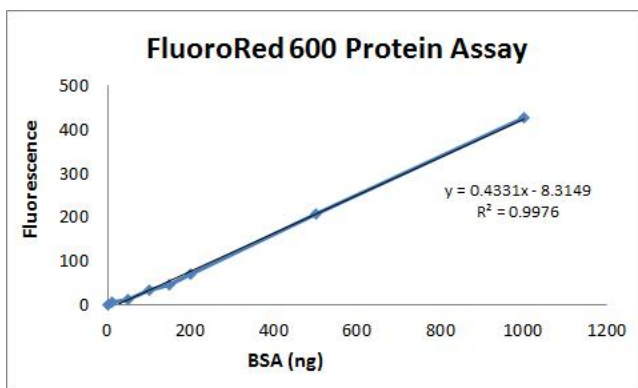


Fig1

## ITEM(S) SUPPLIED

Description	Cat. # 786-1595 (100 microwell assays)	Cat. # 786-1596 (500 microwell assays)
FluoroRed 600 Dye	1 vial	1 vial
DMF	2 ml	2 ml
FluoroRed 600 Reaction Buffer [10X]	10 ml	50 ml
Bovine Serum Albumin (BSA) Standard [2mg/ml]	1 ml	5 ml

## STORAGE CONDITIONS

The kit is shipped with blue ice. Store it at -20°C in dark, upon arrival. When stored and used as recommended, the reagent is stable for 6 months.

## IMPORTANT INFORMATION

- Bring all the kit components to room temperature before use.
- The dye binds to amine groups, therefore the standards and samples to be tested should not be in tris-buffer, glycine or another amine group containing buffer or ammonium containing salts.
- FluoroRed 600 Dye is light sensitive, so make and store the FluoroRed 600 Dye Reaction Reagent protected from light.
- For quantifying very small amount of protein samples (10-100 ng) use siliconized tubes and pipette tips for preparing standards and samples.

## ADDITIONAL ITEMS REQUIRED

- Black microtiter plate
- Fluorescence microplate reader or Fluorimeter
- Multichannel pipette

## PREPARATION BEFORE USE

1. Add 60 µl of DMF to FluoroRed 600 Dye vial (Cat. # 786-1595) and 300 µl DMF to FluoroRed 600 Dye vial (Cat. # 786-1595). Mix well protected from light. Prepare one time use aliquots of dye solution and store them at -20°C protected from light. When stored as recommended, the dye is stable for 6 months.
2. Prepare 1 X FluoroRed 600 Reaction Buffer from FluoroRed 600 Reaction Buffer [10X] by adding FluoroRed 600 Reaction Buffer [10X] to deionized water in ratio 1:9 (eg: Add 1ml of FluoroRed 600 Reaction Buffer [10X] to 9 ml of deionized water to make 1 X solution).
3. Prepare FluoroRed 600 Dye Reaction Reagent by adding 12.5 µl of dye solution to 2.5 ml of 1 X FluoroRed 600 Reaction Buffer.

**NOTE:** *Prepare the Reagent protected from light and prepare immediately before use.*

**NOTE:** *Prepare only the required amount of reagent needed for assay as it is not stable for storage.*

## PROTOCOL

1. Prepare BSA standard as below

**NOTE:** *For minimizing interference, it is advised to prepare the appropriate diluted protein standard in the same diluent used for the test protein sample.*

**NOTE:** *Samples and BSA standard can be prepared in 1X FluoroRed 600 Reaction Buffer or any other amine free buffer with pH8.*

**BSA standard (10 ng-200 ng)**

Prepare 2µg/ml of BSA stock solution by adding 4µl of 2 mg/ml BSA to 1.996 ml of diluent. Prepare further dilutions as below

Bovine Serum Albumin (BSA) Standard [2µg/ml] (µl)	Diluent (µl)	Standard Concentration (µg/ml)	Final amount of BSA in reaction(ng)
300	-	2	200
225	75	1.5	150
150	150	1	100
75	225	0.5	50
15	285	0.1	10
-	300	0	

**NOTE:** This is just an example. One can make their own standard as per requirement as the linear detection range of the assay is 10ng-1 µg/ml (Fig.1)

**Protocol for Standard Microplate or Microwell Assay**

1. Add 100 µl of BSA standard or sample per well in duplicate set to the black microtiter well plate in duplicate set. Add 100 µl of diluent per well in duplicate as a blank.
2. Add 100 µl of FluoroRed 600 Dye Reaction Reagent per well and mix the solution well.
3. Incubate the plate in dark on shaker incubator at room temperature for 30 minutes.
4. Read Fluorescence at excitation wavelength of 490±10 nm and emission of wavelength of 600±10 nm on fluorescence microplate reader.
5. Plot the standard curve for BSA and quantify unknown protein from standard.

**Protocol for Standard Test Tube (1 ml) Assay**

We recommend that the assays are performed in duplicate. This can be performed in two ways. Either dilute the reaction from microplate assay upto 1 ml with 1 X FluoroRed 600 Reaction Buffer or increase the amount of sample and FluoroRed 600 Dye Reaction Reagent proportionately as below.

1. Add 500 µl of BSA standard or sample per well in duplicate set to the black microtiter well plate in duplicate set. Add 100 µl of diluent per well in duplicate as a blank.
2. Add 500 µl of FluoroRed 600 Dye Reaction Reagent per well and mix the solution well.
3. Incubate the plate in dark on shaker incubator at room temperature for 30 minutes.
4. Read Fluorescence at excitation wavelength of 490±10 nm and emission of wavelength of 600±10 nm on fluorimeter.

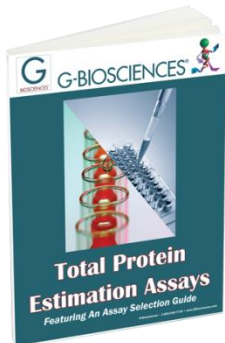
5. Plot the standard curve for BSA and quantify unknown protein from standard.

### **INTERFERENCE TO PROTEIN ASSAY**

The amine buffers such as glycine, Tris and ammonium salts should not be used during reaction as the FluoroRed 660 Dye binds to the amine groups and thus not suitable.

### **RELATED PRODUCTS**

Download our Protein Assays Handbook.



<http://info.gbiosciences.com/complete-protein-assay-guide>

For other related products, visit our website at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.





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