

QuantiFluo™ Protein Assay Kit (QFPR-200)

Quantitative Fluorimetric Determination of Protein/Peptide Concentration

DESCRIPTION

The protein or peptide is known as the "building blocks of life" and is one of the most important macromolecules in life science. Protein determination is a very common practice. Simple, direct and automation-ready procedures for measuring protein or peptide concentration are very desirable. BioAssay Systems' QuantiFluo™ protein assay kit is based on an improved o-phthalaldehyde method. This reagent reacts with primary amines in protein or peptide and forms a blue fluorescent product, allowing detection of nanograms of proteins. The fluorescence intensity ($\lambda_{ex}/\lambda_{em} = 360/450\text{nm}$) is proportional to the protein concentration in the sample.

KEY FEATURES

Fast and sensitive. Assay is completed within a few minutes. Linear detection range of 0.05 - 200 $\mu\text{g/mL}$ BSA.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Protein determination in various biological samples.

KIT CONTENTS

Reagent: 20 mL

Standard: 1 mL 1mg/mL BSA

Storage conditions: This product is shipped at room temperature. For long-term storage, keep kit at -20°C . Shelf life of 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE FOR 96-WELL PLATE READER

Use black flat-bottom 96-well plates. Prior to assay, bring all reagents to room temperature.

Sample preparation. Tissue (20 mg) or cells (2×10^6) can be homogenized in 200 μL ice-cold water, followed by centrifugation at 14,000 rpm for 5 min. Use clear supernatant for assay. Samples not measured on the same day can be stored frozen at -20°C .

Note: (1). This assay is compatible with most detergents, chelators and buffer components. Primary amine-containing buffers (e.g. Tris, glycine) should be avoided, if possible. For best results, include the same concentration of the sample buffer in the standards and blank. (2). If the sample protein concentration is higher than 200 $\mu\text{g/mL}$, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

1. **Standards.** Prepare 200 μL 200 $\mu\text{g/mL}$ BSA standard Premix by mixing 40 μL 1mg/mL Standard and 160 μL H_2O . Dilute standards as follows.

No	Premix + H_2O	Standard ($\mu\text{g/mL}$)
1	100 μL + 0 μL	200
2	50 μL + 50 μL	100
3	25 μL + 75 μL	50
4	0 μL + 100 μL	0

Transfer 10 μL standards into separate wells of the plate.

Transfer 10 μL of each sample in separate wells of the plate.

2. **Assay.** Add 90 μL Reagent to all wells. Immediately tap plate to mix. Measure fluorescence intensity at 360/450nm on a plate reader. It is best to read all samples at the same time interval after mixing.

Note: The standard protocol uses a Sample:Reagent ratio of 1:9. Higher sensitivity can be achieved by using higher sample volume (e.g. 1:1 or 5:1).

CALCULATION

Plot the protein standard curve and determine its Slope. The protein concentration of a Sample is calculated as

$$[\text{Protein}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{\text{Slope}} \quad (\mu\text{g/mL})$$

where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and the blank (i.e. #4 H_2O), respectively.

ASSAY PROCEDURE FOR HANDHELD FLUORIMETER (CAT#: FL360450)

1. **Standard.** Prepare 100 $\mu\text{g/mL}$ by mixing 10 μL of the provided 1 mg/mL BSA standard with 90 μL H_2O or sample buffer.

2. In separate mini-glass tubes (cat#: MGLTB100), add 10 μL H_2O or sample buffer (Blank), 10 μL 100 $\mu\text{g/mL}$ Standard, and 10 μL Sample. Then add 90 μL Reagent to each tube and mix. Incubate for 5 min in the dark.

3. Switch on the reader. To calibrate the reader, place the "Blank" tube into the sample holder. Press "Calibrate", "Assay 1", then "Blank". Reader starts Measuring.

Press "<-Std ->", until the window shows "100.00".

Place the 100 $\mu\text{g/mL}$ Standard into the Sample holder. Press "Measure". The reader shows "Calibrate Finished". Press "Return".

4. **Measure.** Place the sample tube into the Sample Holder.

Press "Measure" → "Assay 1" → "Measure".

The Protein concentration ($\mu\text{g/mL}$) will be displayed in the window. Record the data, or press "Save" to save the data for later retrieval. Press "Return" and then "Measure" for the next sample.

Assay Performance

Linear detection range: 0 to 200 $\mu\text{g/mL}$ (BSA)

Detection limit: 50 ng/mL

Typical precision (CV%): <5%.

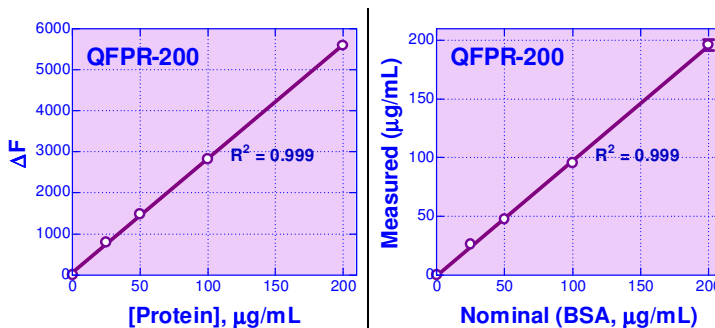
MATERIAL REQUIRED BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, black flat bottom 96-well plates and plate reader.

RELATED PRODUCTS

Handheld Fluorimeter 360/450nm: cat# FL360450.

Mini Glass Tubes for handheld reader (cat# MGLTB100): 100 tubes/bag; Qty of 2 needed for each QFPR-200 kit).



Left: standard curve performed on a 96-well plate reader (Spectramax M2);
Right: correlation plot obtained on handheld fluorimeter (cat#: FL360450).

LITERATURE

1. Kutchai H, Geddis LM (1977). Determinations of protein in red cell membrane preparations by o-phthalaldehyde fluorescence. *Anal Biochem.* 77:315-9.
2. Robrish SA, et al (1978). The use of the o-phthalaldehyde reaction as sensitive assay for protein and to determine protein in bacterial cells and dental plaque. *Anal Biochem.* 84:196-204.
3. Joys TM, Kim H (1979). o-Phthalaldehyde and the fluorogenic detection of peptides. *Anal Biochem.* 94:371-7.