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 ($\lambda_{ex}/_{em}$ = 360/450nm) 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 1.3 - 1000 µg/mL BSA.

Lysis Buffer Compatible. This improved and optimized single reagent works directly with various lysis buffer. Ideal for determining protein concentrations in cell or tissue lysates.

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

For quantitative determination of protein in various biological samples.

KIT CONTENTS

Reagent: 20 mL **Standard:** 100 µL 20 mg/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

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 1000 µg/mL, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

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

Sample preparation. Cultured cells can be assayed directly in a black culture plate. Remove culture media, wash cells 3 times with 1x phosphate-buffered saline (PBS). Alternatively cells can be homogenized in a modified RIPA lysis buffer consisting of: 150 mM NaCl, 5 mM EDTA (pH 8.0), 10 mM HEPES (pH 7.5), 1% NP-40, 0.5% Sodium deoxycholate, 0.5% SDS. (It is preferable to avoid RIPA buffers containing Tris or other primary amines since primary amine increases background fluorescence). After removing media, add enough of 95°C preheated lysis buffer to cover the entire plate (1-1.5 mL for T-75, 500 µL for T-25). Thoroughly coat cells, then use a rubber policeman to detach adherent cells. Sonicate until sample is clear, then heat at 95°C for 10 min. Centrifuge at 1800 x g for 5 min. If debris is present, repeat the sonication, heating, and centrifuge steps until the centrifuged sample no longer leaves debris.

Tissue (20 mg) can be homogenized in 200 µL ice-cold water or a lysis buffer, followed by centrifugation at 14,000 rpm for 5 min.

Samples not measured on the same day can be stored frozen at -80°C.

1. *Standards and Samples*. Prepare a 1000 µg/mL Standard Premix by mixing 10 µL of the 20 mg/mL Standard and 190 µL of H_2O. Dilute the Standard Premix in H_2O as shown in the table. Transfer 10 µL standards into separate wells of the plate.

   Transfer 10 µL of each sample in separate wells of the plate. *Note*: if cell or tissue lysate samples are in a buffer, use the same concentration of buffer instead of H_2O for all standard dilution steps.

No	Premix + H_2O	Standard (µg/mL)
1	100 µL + 0 µL	1000
2	60 µL + 40 µL	600
3	30 µL + 70 µL	300
4	0 µL +100 µL	0

2. *Assay*. Add 90 µL Reagent to all wells. Immediately tap plate to mix. Incubate 5 min at room temperature. 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: 1. 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). 2. For cells cultured in 384-well plates, add 40 µL Reagent to assay wells.

CALCULATION

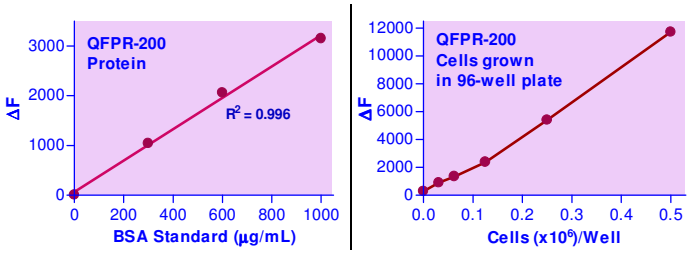
Plot the protein standard curve and determine its Slope. The protein concentration of a Sample is calculated as

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

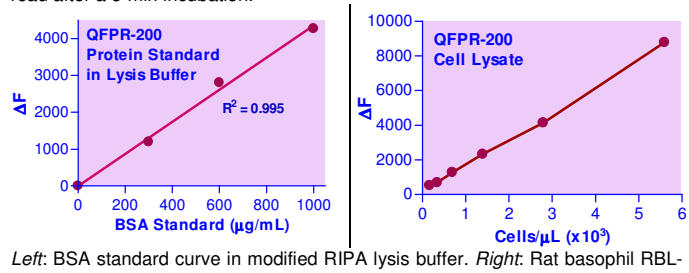
where F_{SAMPLE} and F_{BLANK} are the fluorescence intensity values of the Sample and the blank (i.e. #4 H_2O), respectively.

MATERIAL REQUIRED BUT NOT PROVIDED

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



Left: BSA standard curve. *Right*: Human liver HepG2 cells were plated in a black 96-well tissue culture plate. After overnight culture, cells were washed 3 times with 200 µL PBS. 90 µL Reagent were added to each well. Fluorescence was read after a 5-min incubation.



Left: BSA standard curve in modified RIPA lysis buffer. *Right*: Rat basophil RBL-2H3 cells were homogenized in modified RIPA lysis buffer following the protocol. The resulting samples were used for assay.

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 asensitive 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.