Protein Assay Kit (Fluorometric)

LS-K325-200 (200 Tests) • **Store at -20°C**

Introduction

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. LSBio's protein assay kit is based on an improved ophthalaldehyde 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 0.05 - 200 µg/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.

Components

Component	K325-200
	200 Tests
Reagent	20 mL
Standard (1mg/mL BSA)	1 mL

Materials Not Supplied

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

Storage

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

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Assay Procedure

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 ($2x10^6$) 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 µg/mL, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

Procedure

1. Standards. Prepare 200 µL 200 µg/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 (µg/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).

Calculations

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

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

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

Assay Procedure for Handheld Fluorimeter

1. Standard. Prepare 100 µg/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, add 10 µL H_2O or sample buffer (Blank), 10 µL 100 µg/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 µg/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 (µg/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.

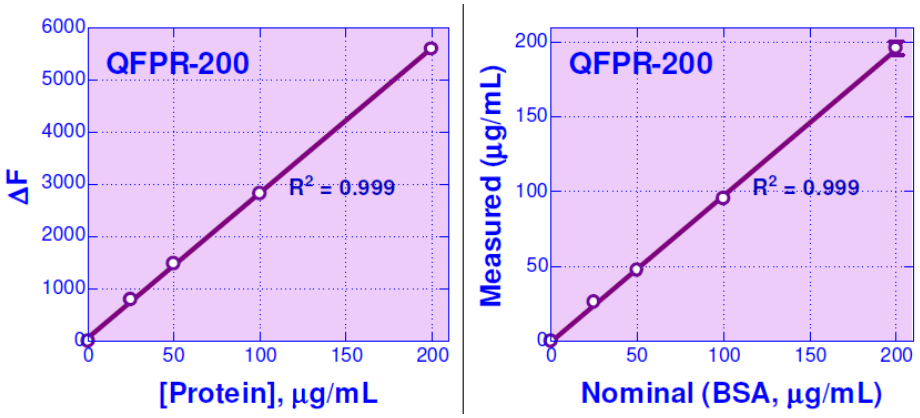
Assay Performance

Linear detection range: 0 to 200 µg/mL (BSA)

Detection limit: 50 ng/mL

Typical precision (CV%): <5%.

Sample Data



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

Version: V.08.09.2018