QuantiChrom™ Protein Assay Kit (QCPR-500)

Bradford Colorimetric Protein Determination at 595 nm

DESCRIPTION

The protein is known as the "building blocks of life" and is one of the most important macromolecules in life science. Proteins are polypeptides made up of amino acids and play various key roles in all aspects of biology. Protein quantitation is a very common practice for life scientists.

Simple, direct and automation-ready procedures for measuring protein concentration are very desirable. BioAssay Systems' QuantiChrom™ protein assay kit is based on an improved Coomassie Blue G method. The dye forms a blue complex specifically with protein, and the intensity of color, measured at 595nm, is directly proportional to the protein concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples and exhibits increased sensitivity towards peptides.

APPLICATIONS

Direct Assays: total protein concentration.

KEY FEATURES

Sensitive and accurate. Use 10 µL samples. Detection range 0.06 – 1.0 mg /mL protein in 96-well plate assay.
Simple and high-throughput. The "mix-and-read" procedure involves addition of a single working reagent and reading the optical density. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.
Low interference. Glucose, Tris, vitamins, and amino acids, DNA, RNA, salts, EDTA (< 12 mM), phenol (< 50 mM), urea (< 0.6 M), Triton (< 0.1%) and SDS (< 0.1% SDS) do not interfere in the assay.
Versatility: assays can be executed in 96-well plate or cuvet.

KIT CONTENTS (500 tests in 96-well plates)

Reagent: 20 mL 5 x concentrate
Protein standard: 1 mL 1.0 mg/mL BSA

Storage conditions. The kit is shipped at room temperature. Store the reagent at 4°C and standard at -20°C, respectively. Shelf life: 12 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.

PROCEDURES

Reagent Preparation:

Prepare enough working reagent by adding 1 vol of the 5 x Reagent to 5 vol of distilled water. Bring reagent to room temperature before use.

Procedure using 96-well plate:

1. Dilute standard as shown in the Table. Transfer 10 µL diluted Standards and diluted sample in duplicate wells of a clear bottom 96-well plate. Store diluted standards at -20°C for future use.

No	Premix + H_2O	Vol (µL)	BSA (mg/mL)	BSA (µg/10 µL)
1	100µL + 0µL	100	1.0	10
2	80µL + 20µL	100	0.8	8
3	60µL + 40µL	100	0.6	6
4	40µL + 60µL	100	0.4	4
5	30µL + 70µL	100	0.3	3
6	20µL + 80µL	100	0.2	2
7	10µL + 90µL	100	0.1	1
8	0µL + 100µL	100	0	0

2. Add 200 µL working reagent and tap lightly to mix.
3. Measure OD at 570-630nm (peak 595nm).

Procedure using cuvette:

1. Prepare standards as in the 96-well plate assay. Transfer 50 µL diluted Standards and 50 µL samples to cuvets.
2. Add 1000 µL working reagent and tap lightly to mix.
3. Measure OD at 570-630nm (peak 595nm).

CALCULATION

Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard concentrations. Use the standard curve to determine the sample protein concentration.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories.

Procedure using 96-well plate:

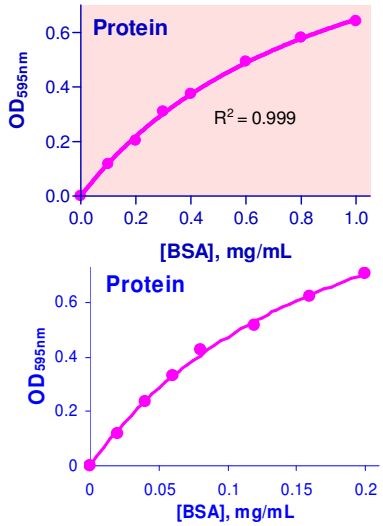
Blank 96-well plates (e.g. Corning Costar).
Plate reader for 96-well plate.

Procedure using cuvette:

Cuvets and spectrophotometer.

GENERAL CONSIDERATIONS

If protein concentration is > 1 mg/mL, dilute samples in distilled water, and use OD values that lie within the calibration curve to calculate the sample protein concentration. Reading can be performed as soon as the reagent and sample are mixed. High sensitivity can be achieved by adding 50 µL sample to 200 µL Reagent (detection range 3 – 200 µg/mL).



Standard Curve in 96-well plate. *Upper*: standard protocol *Lower*: 50 µL sample plus 200 µL Reagent.

PUBLICATIONS

1. Sharifuzzaman SM, et al (2011). Subcellular components of probiotics Kocuria SM1 and Rhodococcus SM2 induce protective immunity in rainbow trout (Oncorhynchus mykiss, Walbaum) against Vibrio anguillarum. Fish Shellfish Immunol. 30(1):347-53.
2. Cucchiarini M et al (2011). Metabolic activities and chondrogenic differentiation of human mesenchymal stem cells following recombinant adeno-associated virus-mediated gene transfer and overexpression of fibroblast growth factor 2. Tissue Eng Part A. 17(15-16):1921-33.
3. Olsen AS et al (2010). Limb regeneration is impaired in an adult zebrafish model of diabetes mellitus. Wound Repair Regen. 18(5):532-42. Assay: Protein in rat tissue.