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User's Manual and Instructions

Bradford Protein Assay Kit (Z5030028)

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. Biochain's Bradford 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. Reagent is light sensitive and should be stored at 4°C in the provided amber bottle for light protection. The standard should be stored at -20°C. Shelf life: 12 months.

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). The signal is stable for about 60 min.

Procedure using cuvette:

1. Prepare standards as in the 96-well plate assay. Transfer 20 μL diluted Standards and 20 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, or fit the standard curve using the equation $\mathbf{y = a \cdot x / (b+x)}$. The protein concentration of Sample is calculated as

$$= \frac{\Delta OD_{SAMPLE} \times \mathbf{b}}{\mathbf{a} - \Delta OD_{SAMPLE}} \times n \quad (\%)$$

$\Delta OD_{SAMPLE} = (OD_{SAMPLE} - OD_{BLANK})$. *n* is the dilution factor (see below).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories.

Procedure using 96-well plate:

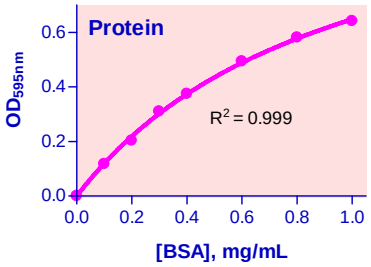
Blank 96-well plates (e.g. Corning Costar).
Plate reader for 96-well plate.

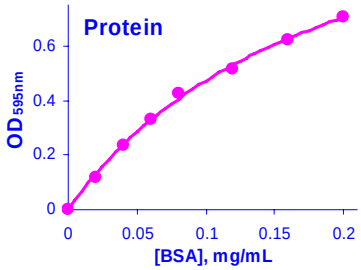
Procedure using cuvette:

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).





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

LITERATURE

1. Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-354.

2. Friedenauer, S. and Berlet, H.H. (1989). Sensitivity and variability of the Bradford protein assay in the presence of detergents. Anal. Biochem. 178: 263-268.

3. Stoscheck, C. M. (1990). Increased uniformity in the response of the Coomassie Blue G protein assay to different proteins. Anal. Biochem. 184: 111-116.