

Technical Manual

ChromaDazzle Protein Assay Kit

Catalogue Code: BA0168

Pack Size: 500 assay

Research Use Only

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. The Assay Genie ChromaDazzle 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 ₂ O	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 μ L diluted Standards and 50 μ L samples to cuvetts
2. Add 1000 μ L 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).

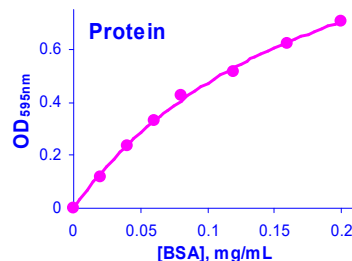
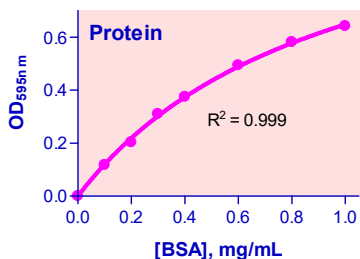
Plate reader for 96-well plate.

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 μ L sample to 200 μ L Reagent (detection range 3 – 200 μ g/mL).



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

PUBLICATIONS

1. Sharifuzzaman, S.M. and Austin, B. (2009). Kocuria SM1 controls vibriosis in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *J Appl Microbiol.* 108(6): 2162 – 2170.
2. Stefanidis A., et al. (2009). The role of thermogenesis in antipsychotic drug-induced weight gain. *Obesity (Silver Spring)*. 17(1):16-24.
3. Verty, A.N. et al. (2009). The effects of rimonabant on brown adipose tissue in rat: implications for energy expenditure. *Obesity (Silver Spring)*. 17(2):254-61.

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