TECHNICAL MANUAL

Bradford Protein Colorimetric Assay Kit

- **SKU CODE:** MAES0126
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to measure total protein (TP) content in serum, plasma, animal tissue, plant tissue, and cell samples.

2. Detection principle

Coomassie brilliant blue G-250 is red in its free state and has maximum absorbance at 465 nm. When Coomassie brilliant blue G-250 binds to protein, the complex exhibits maximum absorbance at 595 nm. The absorbance value is directly proportional to the protein content, so the total protein concentration can be calculated directly by measuring the OD value at 595 nm.

3. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Extraction Solution	110 mL x 1 vial	110 mL x 5 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent Stock Solution	6 mL x 1 vial	30 mL x 1 vial	2-8°C, 12 months, shading light
Reagent 3	1 mg Standard	1 mg x 2 vials	1 mg x 5 vials	RT, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

4. Materials prepared by users

Instruments:

Microplate reader (550 nm-630 nm, optimum wavelength: 595 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge.

Reagents:

Double distilled water

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use.

2. **Preparation of chromogenic agent:** For each well, prepare 250 µL of chromogenic agent by thoroughly mixing 50 µL of chromogenic agent stock solution with 200 µL of double distilled water. Store at 2-8°C for 7 days protected from light.
3. **Preparation of 1 mg/mL standard solution:** Dissolve one vial of 1 mg standard with 1 mL double distilled water and mix well.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mg/mL standard solution with double distilled water to create a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/mL.

6. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one month.

Tissue samples:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in Extraction Solution.

3. Homogenize 20 mg tissue in 180 µL Extraction Solution with a Dounce homogenizer at 4°C.

4. Centrifuge at 10,000 x g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation: 1 x 10^6 cells).

2. Wash cells with Extraction Solution.

3. Homogenize 1 x 10^6 cells in 300-500 µL Extraction Solution with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000 x g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

Human serum: 90-110

Human plasma: 90-110

Rabbit serum: 90-110

Rat plasma: 90-110

Chicken serum: 90-110

10% Mouse kidney tissue homogenate: 15-20

10% Mouse lung tissue homogenate: 15-20

10% Rat spleen tissue homogenate: 15-20

10% Rat heart tissue homogenate: 15-20

10% Rat liver tissue homogenate: 20-25

10% Avocado mesocarp tissue: 3-6

10% Oyster mushroom fruiting body tissue: 1

10% Cucumber mesocarp tissue: 1

10% Carrot cortical tissue: 1

10% Banana mesocarp tissue: 1

10% Chili pepper mesocarp tissue: 1

293T: 1

HL-60: 1

Note: The diluent is Extraction Solution. For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

7. The key points of the assay

Prevent the formation of bubbles when adding liquid to the microplate.

8. Operating steps

1. Standard tube: Add 10 µL of standard solution with different concentrations to the well. Sample tube: Add 10 µL of sample to the well.
2. Add 250 µL of chromogenic agent to each well.
3. Mix fully with microplate reader for 10 s and incubate at room temperature for 10 min.
4. Measure the OD value at 595 nm with microplate reader.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using the absolute OD value of standards and corresponding concentrations as y-axis and x-axis, respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

For sample:

TP content (mg/mL) = (ΔA_595 - b) ÷ a × f

[Note]

f: Dilution factor of sample before test.

ΔA_595: OD_{Sample} - OD_{Blank}.

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Recovery

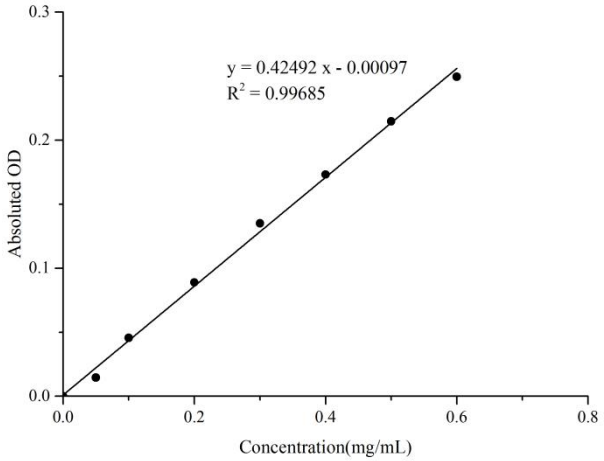
Three samples of high, middle, and low concentrations were tested 6 times in parallel for each concentration to obtain an average recovery rate of 104%.

Sensitivity

The analytical sensitivity of the assay is 0.046 mg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only.



11. Appendix II Example Analysis

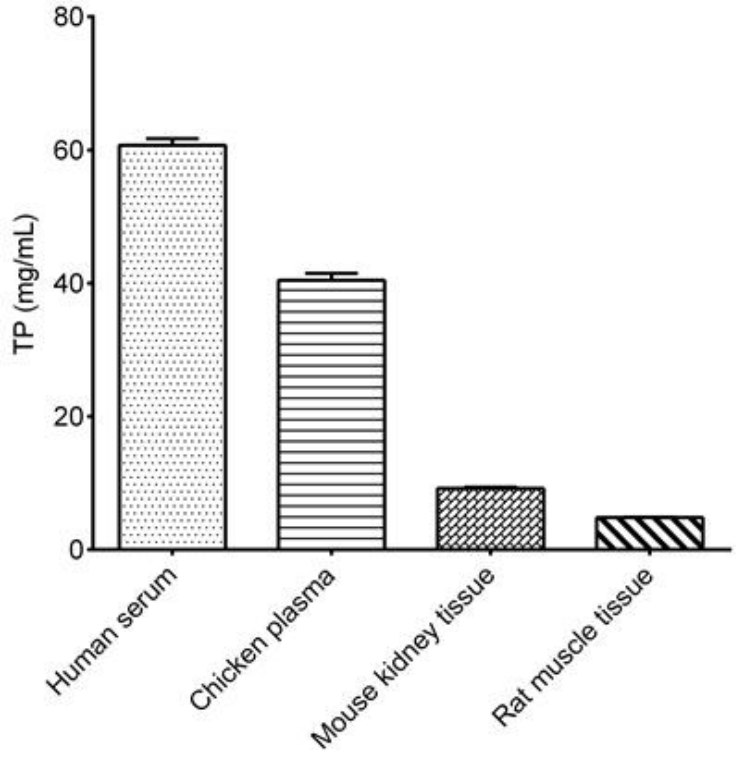
Example analysis:

Dilute human serum with Extraction Solution 100-fold, take 10 µL of diluted sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.3727x + 0.0043, the average OD value of the sample is 0.550, the average OD value of the blank is 0.319, and the calculation result is:

TP content (mg/mL) = (0.550 - 0.319 - 0.0043) ÷ 0.3727 × 100 = 60.83 mg/mL

Detect human serum (diluted 100-fold), chicken plasma (diluted 100-fold), 10% mouse kidney tissue homogenate (diluted 20-fold), and 10% rat muscle tissue homogenate (diluted 20-fold) according to the protocol. The results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.