TECHNICAL MANUAL

Albumin (ALB) Colorimetric Assay Kit (Bromocresol Green)

- **SKU CODE:** MAES0065
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure absorbance at 630 nm

2. Intended use

This kit can be used to measure albumin (ALB) content in serum, plasma, and cell culture supernatant samples.

3. Detection principle

Bromocresol green (BCG) is widely used as a protein staining agent. BCG combines with albumin at pH 4.0-4.2 to form an albumin-BCG complex, causing the color to change from yellow to green. The depth of color is proportional to the concentration of albumin. The albumin content in serum can be calculated indirectly by measuring the OD value at 630 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500Assays)	Storage
Reagent 1	Chromogenic Agent Stock Solution	3 mL x 1 vial	6 mL x 1 vial	30 mL x 1 vial	2-8 °C, 12 months, protect from light
Reagent 2	20 g/L Standard Solution	1.2 mL x 1 vial	1.2 mL x 2 vials	12 mL x 1 vial	-20 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (620-640 nm, optimum wavelength: 630 nm), Micropipettor, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Keep 20 g/L standard solution on ice during use. Allow chromogenic agent stock solution to equilibrate to room temperature before use.
2. **Preparation of chromogenic working solution:** For each well, prepare 250 µL of chromogenic working solution by mixing 50 µL of chromogenic agent stock solution with 200 µL of double distilled water. Mix well. The chromogenic working solution should be prepared fresh before use.
3. Take 20 g/L standard solution from -20 °C and place on ice to thaw slowly. It is recommended to aliquot the 20 g/L standard solution to avoid repeated freeze-thaw cycles.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 20 g/L standard solution with double distilled water to create a serial dilution. The recommended dilution gradient is as follows: 0, 1, 2, 3.5, 5, 8, 12, 15 g/L.

7. Standard dilution table

Item	1	2	3	4	5	6	7	8
Concentration (g/L)	0	1	2	3.5	5	8	12	15
20 g/L standard solution (µL)	0	10	20	35	50	80	120	150
Double distilled water (µL)	200	190	180	165	150	120	80	50

8. Sample preparation

Sample preparation:

Serum (plasma) and cell culture supernatant: detect directly. If not tested on the same day, serum or plasma can be stored at -80 °C for one month.

Dilution of sample:

The recommended dilution factors for different samples are as follows (for reference only):

Human serum: 8-15 fold

Human plasma: 8-15 fold

HepG2 supernatant: 1 fold

Mouse plasma: 8-15 fold

Rat serum: 8-15 fold

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For other sample types, please perform a pretest to confirm the appropriate dilution factor.

9. Key points of the assay

1. Prevent the formation of bubbles in the microplate.
2. Standards should avoid repeated freeze-thaw cycles.
3. Chromogenic working solution should be stored protected from light.

10. Operating steps

1. Standard wells: Add 10 µL of standard with different concentrations to the wells.
   Sample wells: Add 10 µL of sample to the wells.
2. Add 250 µL of chromogenic working solution to each well.
3. Incubate for 10 minutes at room temperature.
4. Measure the OD value of each well at 630 nm using a microplate reader.

11. Calculation

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 gives the absolute OD value.

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

Sample calculation:

For serum (plasma) and cell culture supernatant samples:

ALB content (g/L) = (ΔA_630 - b) ÷ a × f

[Note]

ΔA_630: OD_{Sample} - OD_{Blank}

f: Dilution factor of sample before testing

Intra-assay Precision

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

Intra-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (g/L)	0.65	3.50	9.60
%CV	1.8	1.4	1.3

Inter-assay Precision

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

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (g/L)	0.65	3.50	9.60
%CV	4.3	4.8	4.7

Recovery

Three samples of high, medium, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 95%.

Recovery Results

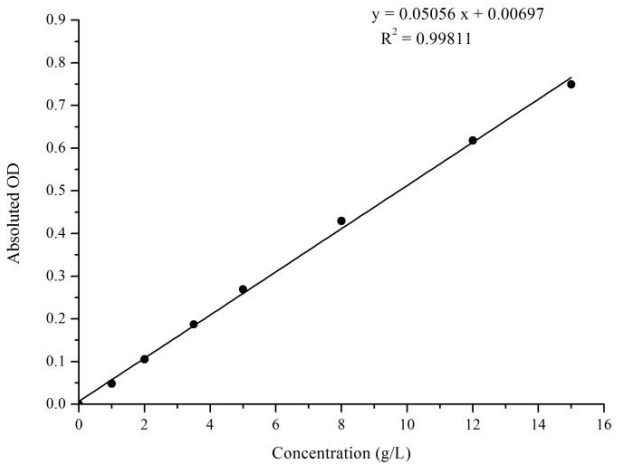
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (g/L)	1.5	4	10.5
Observed Conc. (g/L)	1.5	3.7	9.9
Recovery rate (%)	98	93	94

Sensitivity

The analytical sensitivity of the assay is 0.08 g/L. 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 data

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



Standard curve reference data

Concentration (g/L)	0	1.0	2.0	3.5	5.0	8.0	12	15
Average OD	0.122	0.170	0.227	0.309	0.391	0.552	0.740	0.871
Absolute OD	0	0.048	0.105	0.187	0.269	0.430	0.618	0.749

12. Example Analysis

Example analysis:

Dilute human serum with normal saline at a ratio of 1:9, take 10 µL of diluted sample, and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.0505x + 0.00779

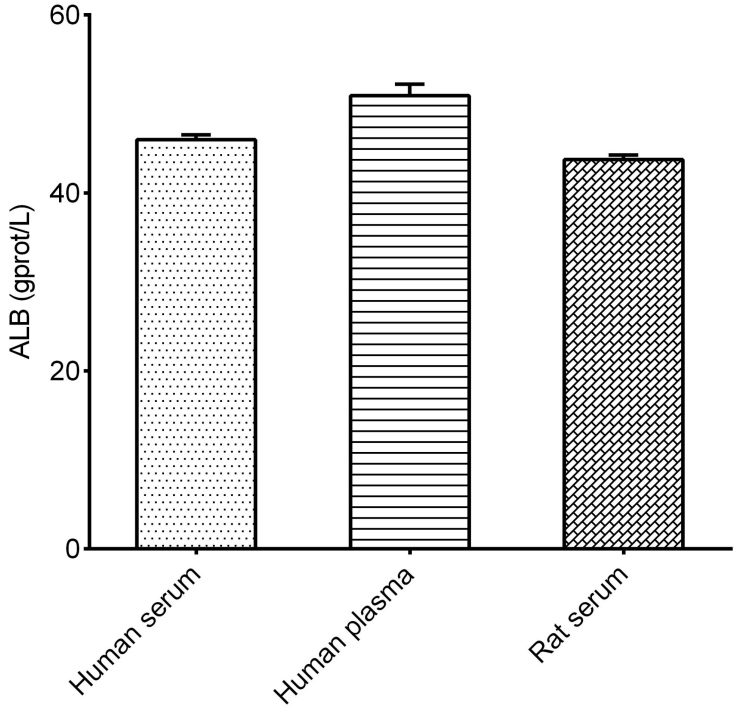
Average OD value of sample: 0.364

Average OD value of blank: 0.113

Calculation result:

ALB content (g/L) = (0.364 - 0.113 - 0.0079) ÷ 0.0505 × 10 = 48.75 g/L

Testing human serum (diluted 10-fold), human plasma (diluted 10-fold), and rat serum (diluted 10-fold) according to the protocol yields the following results:



13. 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 pretest 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.