# Albumin (ALB) Colorimetric Assay Kit (Bromocresol Green Method)

Catalog No: E-BC-K057-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.50 g/L

Detection range: 0.50-70 g/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

# General information

## ▲Intended use

This kit can be used to measure the Albumin (ALB) in serum (plasma) samples.

## ▲Background

Albumin is the most abundant plasma protein, with a molecular weight of 66.5 kDa, synthesized in the liver at a rate of 9-12 grams per day and regulated by insulin, amino acid intake and low colloidal osmotic pressure. Changes in urine and serum albumin levels are predictors of diabetic nephropathy, cardiovascular disease, liver disease and sepsis.

## ▲Detection principle

Bromocresol green (BCG) can combine with the albumin in pH 4.0~4.2 to form an albumin-BCG complex, which is yellowish-green. The depth of yellowish-green is proportional to the concentration of albumin. The serum albumin concentration can be calculated by measuring the OD value at 628 nm.

## ▲Kit components & storage

| Item | Component | Specification | Storage |
|---|---|---|---|
| Reagent 1 | Stock Solution | 60 mL ×1 vial | 2-8℃ , 6 months, shading light |
| Reagent 2 | 40 g/L Standard | 0.3 mL × 1 vial | -20℃ , 6 months |
| Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. | | | |

## ▲Materials prepared by users

### Instruments

Spectrophotometer (628 nm), Micropipettor, Vortex mixer

### Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

### Reagents

Double distilled water, normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

## ▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

## ▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

## ▲The key points of the assay

1. Standard should be avoid repeated freezing and thawing.
2. Reagent 1 working solution should be stored with shading light.

# Pre-assay preparation

## ▲Reagent preparation

1. Take the reagent 2 from -20 ℃ and place on ice to thaw slowly. It is recommended to aliquot the reagent 2 to avoid repeated freezing and thawing.
2. Preparation of reagent 1 working solution:
   Dilute the reagent 1 with double distilled water at a ratio of 1:4. Prepare the fresh solution before use and the unused resgent can be stored at 2-8℃ for 3 days.

## ▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

## ▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.50-70 g/L).

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

| Sample type | Dilution factor |
|---|---|
| Human serum | 1 |
| Human plasma | 1 |
| Mouse plasma | 1 |
| Rat serum | 1 |

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

| Assay protocol | |
|---|---|
| Ambient temperature | 25-30°C |
| Optimum detection wavelength | 628 nm |

**Instructions for the use of transferpettor:**

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

# Assay protocol

## ▲Operating steps

1. Blank tube: add 10 μL of double distilled water into a 5 mL EP tube.
   Standard tube: add 10 μL of 40 g/L Standard into a 5 mL EP tube.
   Sample tube: add 10 μL of Sample into a 5 mL EP tube.
2. Add 2500 μL of reagent 1 working solution into each tube. Mix fully with a vortex mixer and stand at room temperature for 10 min.
3. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 628 nm with 1 cm optical path cuvette.

## ▲Operation table

| | Blank tube | Standard tube | Sample tube |
|---|---|---|---|
| Double-distilled water (μL) | 10 | | |
| 40 g/L Standard (μL) | | 10 | |
| Sample (μL) | | | 10 |
| Reagent 1 working solution (μL) | 2500 | 2500 | 2500 |
| Mix fully with a vortex mixer and stand at room temperature for 10 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 628 nm with 1 cm optical path cuvette. | | | |

## ▲Calculation

$$\text{Albumin content (g/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

**Note:**

$\Delta A_1$: $OD_{Sample} - OD_{Blank}$

$\Delta A_2$: $OD_{Standard} - OD_{Blank}$

f: Dilution factor of sample before test.

c: Concentration of standard, 40 g/L

## ▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

# Appendix I Performance characteristics

| Appendix I Performance characteristics | | | |
|---|---|---|---|
| Detection range | 0.50-70 g/L | Average intra-assay CV (%) | 2.1 |
| Sensitivity | 0.50 g/L | Average inter-assay CV (%) | 4.2 |
| Average recovery rate (%) | 99 | | |

## ▲Example analysis

Take 0.01 mL of human serum and carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.449, the average OD value of the blank is 0.101, the average OD value of the standard is 0.404, the concentration of standard is 40 g/L, and the calculation result is:

$$\text{Albumin content (g/L)} = \frac{0.449-0.101}{0.404-0.101} \times 40 = 45.94 \text{ g/L}$$

# Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

## ▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

## ▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at-80℃ for a month.

## ▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.