(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Catalog No: E-BC-K057-S Specification: 50 assays(48 samples) / 100 assays(96 samples) Measuring instrument: Spectrophotometer (628 nm) Detection range: 0.50-70 g/L

Elabscience[®]Albumin (ALB) Colorimetric Assay Kit (Bromocresol Green Method)

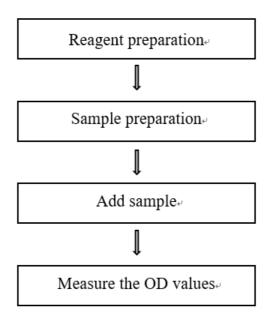
This manual must be read attentively and completely before using this product. If you have any problem, please contact our Technical Service Center for help:

Toll-free: 1-888-852-8623 Tell: 1-832-243-6086 Fax: 1-832-243-6017 Email: techsupport@elabscience.com Website: www.elabscience.com

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

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Intended use

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

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	Component Size 1 (50 assays) (10		Storage
Reagent 1	Stock Solution	30 mL ×1 vial	$60 \text{ mL} \times 1 \text{ vial}$	2-8°C, 12 month, shading light
Reagent 2	40 g/L Standard	0.3 mL ×1 vial	$0.3 \text{ mL} \times 1 \text{ vial}$	-20°C, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (628 nm), Micropipettor, Vortex mixer

Reagents:

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

Reagent preparation

- Keep 40 g/L standard solution on ice during use. Equilibrate stock solution to room temperature before use.
- ② The preparation of stock working solution: For each well, prepare 2500 μL of stock working solution (mix well 500 μL of chromogenic agent stock solution and 2000 μL of double distilled water). The stock working solution should be prepared on spot. Store at 2-8 ℃ for 3 days.
- ③ Take 40 g/L standard solution from -20 °C and place on ice to thaw slowly. It is recommended to aliquot the 40 g/L standard solution to avoid repeated freezing and thawing.

Sample preparation

(1) Sample preparation

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

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

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

Operating steps

- 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 stock 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.

Calculation

The sample:

Serum (plasma) cell culture supernatant sample:

 $\begin{array}{l} Albumin \ content \\ (g/L) = & \underline{\Delta A_1} \\ \underline{\Delta A_2} \times c \times f \end{array}$

[Note]
△A₁: OD_{Sample} – OD_{Blank}.
△A₂: OD_{Standard} – OD_{Blank}.
f: Dilution factor of sample before test.
c: Concentration of standard, 40 g/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3	
Mean (g/L)	8.50	29.50	65.00	
%CV	2.4	2.0	1.9	

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3	
Mean (g/L)	8.50	29.50	65.00	
%CV	4.1	4.3	4.2	

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (g/L)	13	37.5	58
Observed Conc. (g/L)	12.9	37.5	56.8
recovery rate(%)	99	100	98

Sensitivity

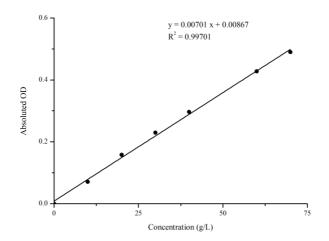
The analytical sensitivity of the assay is 0.50 g/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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), so the standard curve and data are provided as below for reference only:

Concentration (g/L)	0	10	20	30	40	60	70
Average OD	0.098	0.098	0.098	0.098	0.098	0.098	0.098
Absoluted OD	0.168	0.168	0.168	0.168	0.168	0.168	0.168



Appendix II Example Analysis

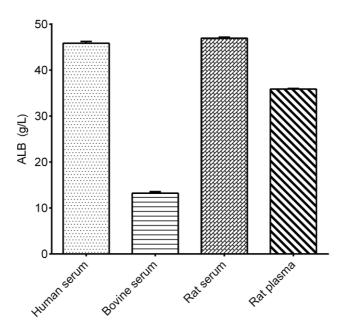
Example analysis:

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

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

Detect human serum, bovine serum, rat serum and rat plasma according to the protocol, the result is as follows:



Statement

- 1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
- 2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
- 3. Protection methods must be taken by wearing lab coat and latex gloves.
- 4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
- 5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
- 6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.