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

Catalog No：E-BC-K057-M

Specification：48T(32 samples)/96T(80 samples)/500Assays(484 samples)

Measuring instrument：Microplate reader（620-640 nm）

Detection range：0.08-15 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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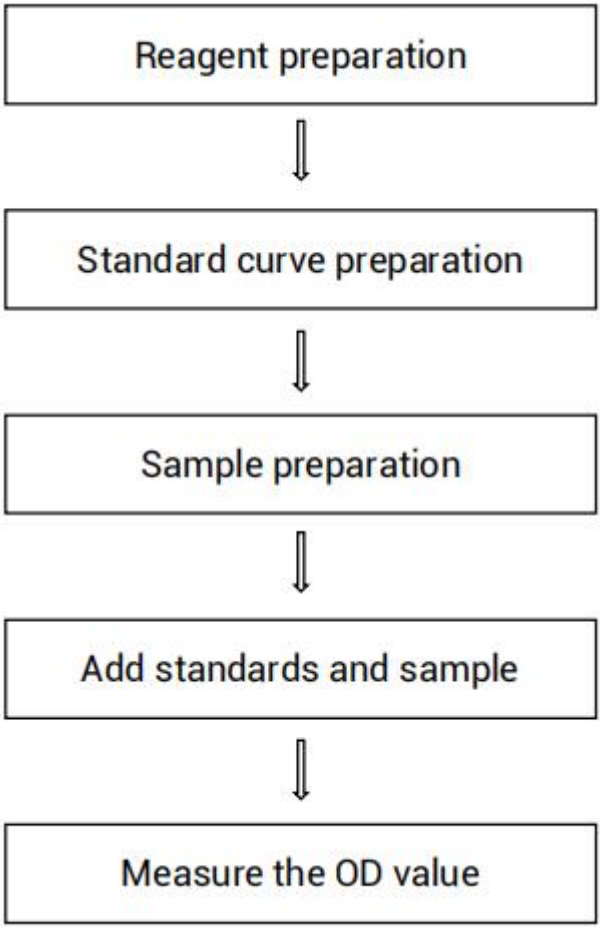
Assay summary



Intended use

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

Detection principle

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

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×1vial	6 mL×1 vial	30 mL×1 vial	2-8℃, 12 months, shading light
Reagent 2	20 g/L Standard Solution	1.2 mL×1 vial	1.2 mL×2 vials	12 mL×1 vial	-20℃, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			
	Sample Layout Sheet	1 piece			

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:

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

Micropipettor, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Keep 20 g/L standard solution on ice during use. Equilibrate chromogenic agent stock solution to room temperature before use.

② The preparation of chromogenic working solution：

For each well， prepare 250 uL of chromogenic working solution (mix well 50 μL of chromogenic agent stock solution and 200 μL of double distilled water). The chromogenic working solution should be prepared on spot.

③ Take 20 g/L standard solution from -20 ℃ and place on ice to thaw slowly. It is recommended to aliquot the 20 g/L standard solution to avoid repeated freezing and thawing

④ The 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 a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3.5, 5, 8, 12, 15g/L. Reference is as follows:

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

Sample preparation

① Sample preparation

Serum (plasma) and cell culture supernatant: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ 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	8-15
Human plasma	8-15
HepG2 supernatant	1
Mouse plasma	8-15
Rat serum	8-15

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 Prevent the formulation of bubbles in the microplate.

② Standard should be avoid repeated freezing and thawing.

③ Chromogenic working solution should be stored with shading light.

Operating steps

① Standard well: add 10 μL standard with different concentration into the wells.
Sample well: add 10 μL of sample into the wells.
② Add 250 μL of the chromogenic working solution to each well.
③ Stand for 10 min at room temperature.
④ Measure the OD value of each well at 630 nm with Microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Serum (plasma) and cell culture supernatant sample:

$$\text{ALB content (g/L)} = (\Delta A_{630} - b) \div a \times f$$

[Note]

ΔA_{630}: $OD_{Sample} - OD_{Blank}$.
f: Dilution factor of sample before test.

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)	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.

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

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 95%.

	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

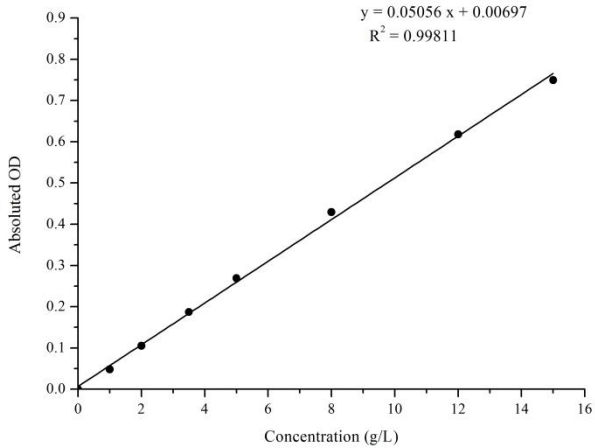
Sensitivity

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.

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

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
Absoluted OD	0	0.048	0.105	0.187	0.269	0.430	0.618	0.749



Appendix Π Example Analysis

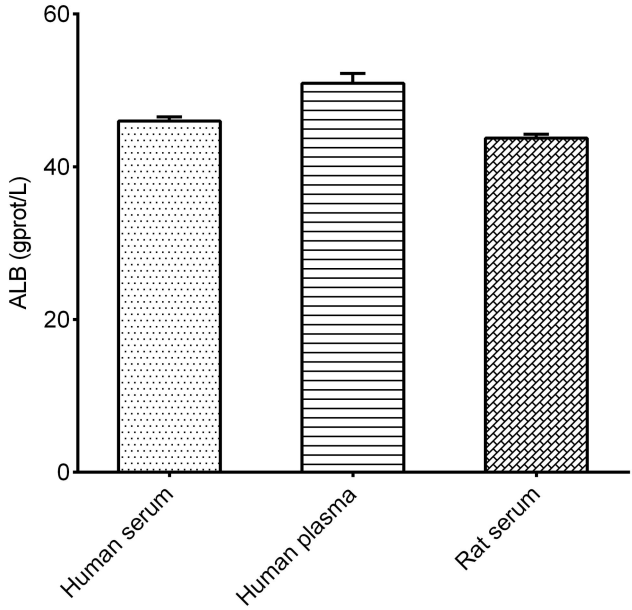
Example analysis：

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

standard curve: y = 0.0505 x +0.00779, the average OD value of the sample is 0.364, the average OD value of the blank is 0.113, and the calculation result is:

$$\underset{(g/L)}{\text{ALB content}} = (0.364 - 0.113 - 0.0079) \div 0.0505 \times 10 = 48.75\ g/L$$

Detect human serum (dilute for 10 times), human plasma (dilute for 10 times), rat serum (dilute for 10 times) 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.