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

Catalog No: E-BC-K057-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.08 g/L

Detection range: 0.08-15 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) content in serum, plasma, cell culture supernatant samples.

▲Background

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

▲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	Specification	Storage
Reagent 1	Chromogenic Agent Stock Solution	6 mL ×1 vial	2-8℃ , 6 months, shading light
Reagent 2	20 g/L Standard Solution	1.2 mL × 2 vials	-20℃ , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader(620-640 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)

▲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. Prevent the formulation of bubbles in the microplate.
2. Standard should be avoid repeated freezing and thawing.
3. Reagent 1 working solution should be stored with shading light.

Pre-assay preparation

▲Reagent preparation

1. The preparation of reagent 1 working solution
   Dilute the reagent 1 with double distilled water at a ratio of 1:4 and mix fully. Prepare the fresh solution before use.
2. 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.

▲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 (0.08-15 g/L).

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).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	630 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

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A, blank well; B-H, standard wells; S1-S80, sample wells.

▲Operating steps

The preparation of standard curve

Dilute 20 g/L BSA 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, 15 g/L.

The measurement of samples

(1) Standard well: add 10 μL standard with different concentration into the wells.
Sample tube: add 10 μL of sample into the wells.

(2) Add 250 μL of the reagent 1 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.

▲Operation table

	Standard well	Sample well
BSA Standard solution with different concentrations (μL)	10	
Sample (μL)		10
Reagent 1 working solution (μL)	250	250
Stand at room temperature for 10 min. Measure the OD values of each tube at 630 nm with Microplate reader.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

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

Note:

y: The absolute OD value of standard

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA_{630}: $OD_{Sample} - OD_{Blank}$

f: Dilution factor of sample before test

▲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

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Detection range	0.08-15 g/L	Average intra-assay CV (%)	1.5
Sensitivity	0.08 g/L	Average inter-assay CV (%)	4.6
Average recovery rate (%)	95		

▲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 table.

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:

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C . 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°C for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4°C . 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°C for a month.

▲Cell culture supernatant

Collect the cell culture supernatant, centrifuge at 10000 g for 10 min at 4°C ,and then take the supernatant to preserve it on ice for detection.

▲ 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.