

(90 × 210mm Size)

〈For Research Use Only〉 Code No. 292-63901 (1,000 Tests)



Albumin to Globulin Ratio Assay BCG method, Biuret method

〔Introduction〕

Blood serum contains two major protein groups: albumin and globulin. The ratio of albumin to globulin (A/G ratio) is calculated from values obtained by direct measurement of total protein and albumin. It represents the relative amounts of albumin and globulins.

A/G assay is able to measure total protein in mouse and human serum by the Biuret method and albumin by the BCG (Bromocresol green) method, and furthermore to calculate A/G ratio. It is a simultaneous multi-sample assay format using a microplate, but measurements can also be made with a test tube.

〔Kit contents〕

(1)	Albumin Chromogen Reagent (Succinate buffer 75mmol/L, pH 4.2 Bromocresol green 0.17mmol/L Detergent)	250mL	1vial
(2)	Total Protein Chromogen Reagent (Copper(II) Sulfate Pentahydrate 12mmol/L Potassium Sodium Tartrate Sodium Hydroxide)	250mL	1vial
(3)	Standard Serum (from Bovine Serum)	For 3mL	1vial
(4)	Albumin Adjustment Buffer (Succinate buffer 75mmol/L, pH 4.2)	25mL	1vial

〔Materials and apparatuses to be prepared〕

- 96wells microplate (transparent type)
- Micropipette
- Plate mixer*
- Microplate reader with 540nm and 630nm wavelength filter
(* if the microplate reader is not equipped.)

〔For test tube method〕

- Test tube
- Pipette
- Spectrophotometer or colorimeter with 540nm and 630nm wavelength filter

〔Assay principle〕

- (1) Albumin determination (BCG method)
Albumin in the sample binds with Bromocresol green (BCG), which produces a blue pigment. Quantitation of albumin in the sample can be made by measurement of the absorbance.
- (2) Total protein determination (Biuret method)
Protein in the sample forms a complex salt with copper ion, and which produces a blue-purple pigment. Quantitation of protein in the sample can be made by measurement of the absorbance.

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Globulin concentration is calculated by subtracting albumin one from total protein one.

〔Preparation of reagents to be used〕

- ① Albumin Chromogen Reagent
The provided reagent is ready-to-use.
- ② Total Protein Chromogen Reagent
The provided reagent is ready-to-use.
- ③ Albumin Adjustment Reagent
The provided reagent is ready-to-use.
- ④ Standard Serum
Standard stock solution is prepared by adding 3mL of distilled water to a vial the provided Standard Serum.
- ⑤ Standard solution (for microplate method)
Each standard solution is prepared by dilution of the prepared standard stock solution.

(1) Albumin

No.	The prepared std. stock solution	Distilled water for dilution	Sampling volume of diluted std.	Albumin concentration *1
1	20 μ L	80 μ L	1 μ L	1.0g/dL
2	40 μ L	60 μ L	1 μ L	2.0g/dL
3	60 μ L	40 μ L	1 μ L	3.0g/dL
4	80 μ L	20 μ L	1 μ L	4.0g/dL
5	1 μ L	—	1 μ L	5.0g/dL
6	Standard No.1 1 μ L + stock solution 1 μ L	—	2 μ L	6.0g/dL

*1 In case of containing 5.0 g/dL albumin in standard serum.

(2) Total protein

No.	The prepared std. stock solution	Distilled water for dilution	Sampling volume of diluted std.	Albumin concentration *2
1	20 μ L	80 μ L	5 μ L	1.6g/dL
2	40 μ L	60 μ L	5 μ L	3.2g/dL
3	60 μ L	40 μ L	5 μ L	4.8g/dL
4	80 μ L	20 μ L	5 μ L	6.4g/dL
5	5 μ L	—	5 μ L	8.0g/dL
6	7.5 μ L	—	7.5 μ L	11.9g/dL

*2 In case of containing 8.0 g/dL total protein in standard serum.

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[Procedure]

(1) Assay in a microplate

Perform the assay in the wells according to the following table scheme.

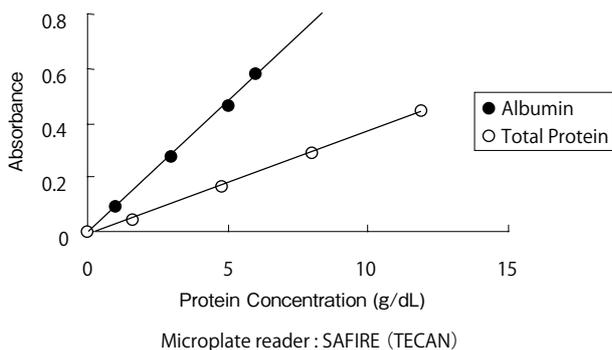
	Albumin			Total protein		
	Test	Standard	Blank	Test	Standard	Blank
Sample	Serum 1 μ L	Std. Soln. 1 μ L	—	Serum 5 μ L	Std. Soln. 5 μ L	Distilled water 5 μ L
Chromogen reagent	250 μ L	250 μ L	250 μ L	250 μ L	250 μ L	250 μ L
	Mix well, and incubate at room temperature for 10 min. Measure the absorbance of wavelength 630nm of the test sample and standard solution with the blank solution as the control within an hour.			Mix well, and incubate at room temperature for 30 min. Measure the absorbance of wavelength 540nm of the test sample and standard solution with the blank solution as the control.		

(2) Assay in a test tube

Perform the assay in a test tube according to the following table scheme.

	Albumin			Total protein		
	Test	Standard	Blank	Test	Standard	Blank
Sample	Serum 20 μ L	Std. Soln. 20 μ L	—	Serum 100 μ L	Std. Soln. 100 μ L	Distilled water 100 μ L
Chromogen reagent	5.0mL	5.0mL	5.0mL	5.0mL	5.0mL	5.0mL
	Mix well, and incubate at room temperature for 10 min. Measure the absorbance of wavelength 630nm of the test sample and standard solution with the blank solution as the control within an hour.			Mix well, and incubate at room temperature for 30 min. Measure the absorbance of wavelength 540nm of the test sample and standard solution with the blank solution as the control.		

[Standard curve]



[Calculation]

$$A/G \text{ ratio} = \frac{\text{(Albumin concentration)}}{\text{(Total protein concentration)} - \text{(Albumin concentration)}}$$

[Performance]

• **Albumin**

(1) Sensitivity

- The absorbance is 0.120 to 0.220, when measuring purified water as a sample.
- The absorbance is 0.480 to 0.810, when measuring standard serum (5.0g/dL albumin) as a sample.

(2) Specificity

The albumin concentration is less than $\pm 12\%$, when measuring the known concentration of control serum as a sample.

• **Total protein**

(1) Sensitivity

- The absorbance is 0.050 to 0.100, when measuring purified water as a sample.
- The absorbance is 0.300 to 0.500, when measuring standard serum (8.0g/dL total protein) as a sample.

(2) Specificity

The total protein concentration is less than $\pm 10\%$, when measuring the known concentration of control serum as a sample.

[Usage Notes]

(1) Sample

- Oxalate and EDTA as a coagulant and sodium fluoride as an anti-glycolysis may not affect the assay. However, heparin should not be used, as it slightly affects the albumin assay.
- Hemolysis slightly affects the albumin assay. Bilirubin may not significantly affect the assay.
- High emulsion serum affects the assay.

(2) Notes on the assay

- Keep the provided reagents under the indicated conditions prior to the expiration date.
- The absorbance is maximum 10 min. after adding Albumin Chromogen Reagent. It is kept for 1 hour.
- The absorbance is maximum 30 min. after adding Total Protein Chromogen Reagent. It is kept for 2 hours.
- The standard curve should be made during each assay occasion.
- Reaction temperature may not affect the total protein assay.
- Adjustment method for high-fat serum
 - 1) In albumin assay, the exact value should be calculated by subtracting the absorbance of sample blank from one of sample Sample blank assay :
Add 5.0mL of Albumin Adjustment Buffer to 0.02mL of the serum. Mix well, and measure the absorbance of wavelength 630nm of the solution with water as a blank.
 - 2) In total protein assay, the exact value should be calculated by subtracting the absorbance of sample blank from one of sample. Sample blank assay :
Add 5.0mL of saline solution to 0.1mL of the serum. Mix well, and measure the absorbance of wavelength 540nm of the solution with water as a blank.
- Standard serum is lyophilized bovine serum. It should not be used for other purposes.
- Total protein chromogen reagent contains 0.3% Copper (II) Sulfate Pentahydrate (760 mg/L as a copper).
- This kit is for research use only, not for diagnostic use. The waste should be processed appropriately.

Expiration date : 1 year after the manufacture

Storage : Store at 2 ~ 10°C

Package : 1,000 tests

[References]

1. Dumas, B. T., Watson, W. A. and Biggs, H. G. : *Clin. Chem. Acta.*, **31**, 87 (1971).
2. Gornall, A. G., Bardawill, C. J. and David, M. M. : *J. Biol. Chem.*, **177**, 751 (1949).

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