Technical Bulletin

BCG (Bromocresol Green) Albumin Assay Kit

Catalog number MAK124

Product Description

Albumin is the most abundant plasma protein in humans. It accounts for ~60% of the total serum protein. Albumin plays important physiological roles, including maintenance of colloid osmotic pressure and binding of key substances such as long-chain fatty acids, bile acids, bilirubin, hematin, calcium, and magnesium. It has antioxidant and anticoagulant effects, acts as a carrier for nutritional factors and drugs, and is an effective plasma pH buffer. Serum albumin is a reliable prognostic indicator for morbidity and mortality, liver disease, nephritic syndrome, malnutrition, and protein-losing enteropathies. High levels are associated with dehydration.

The BCG (Bromocresol Green) albumin assay kit is designed to measure albumin directly without any pretreatment of samples, such as serum, plasma, urine, and biological preparations. The optimized formulation substantially reduces interference by other substances (lipids/other proteins) in the raw samples. It may also be used to measure effects of drugs and other compounds on albumin metabolism.

The kit may be used for cuvette or multiwell plate assays. The multiwell plate assay uses samples as small as 5 µL and can be readily automated as a high-throughput assay for thousands of samples per day.

The procedure involves addition of a single working reagent and a 5 minute incubation. The optimized formulation has greatly enhanced reagent and signal stability. The kit utilizes bromocresol green, which forms a colored complex specifically with albumin. The intensity of the color, measured at 620 nm, is directly proportional to the albumin concentration in the sample.

Components

The kit is sufficient for 250 assays in 96 well plates.

Reagent Catalog Number MAK124A	50 mL
Albumin Standard 5 g/dL Catalog Number MAK124B	1 mL

Storage/Stability

This kit is shipped at room temperature and storage at -20 °C is recommended. For ease of use, the Reagent is stable at and can be stored at 2-8 °C.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagents and Equipment Required but Not Provided.

96 Well Plate Assay

- 96 well flat-bottom plate – It is recommended to use clear bottom plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Cuvette Assay

- Spectrophotometer suitable for 620 nm reading
    - Cuvettes suitable for 620 nm reading

Preparation Instructions

Use ultrapure water for dilutions.

Bring Reagent to room temperature and shake before use.

Dilute Albumin Standard (5 g/dL) in ultrapure water (see Table 1.).

Table 1.

Dilution of Albumin Standard

Std	Albumin Standard	Water	[BSA]
1	100 µL	0 µL	5.0 g/dL
2	80 µL	20 µL	4.0 g/dL
3	60 µL	40 µL	3.0 g/dL
4	40 µL	60 µL	2.0 g/dL
5	30 µL	70 µL	1.5 g/dL
6	20 µL	80 µL	1.0 g/dL
7	10 µL	90 µL	0.5 g/dL
Blank	0 µL	100 µL	0 g/dL

Note: Diluted standards may be stored at -20 °C for future use.

Dilute serum and plasma samples 2-fold with water.

Procedures

96 well plate Assay

1. Transfer 5 µL of diluted Standards, Blank, and diluted Samples to appropriate wells of a clear bottom plate.
2. Add 200 µL of Reagent and tap lightly to mix. Avoid bubbles.
3. Incubate 5 minutes at room temperature and measure absorbance at 570-670 nm (peak absorbance at 620 nm)

Cuvette Assay

1. Transfer 20 µL of diluted Standards, Blank, and diluted Samples to appropriately labeled tubes. Add 1,000 µL of Reagent and tap lightly to mix. Incubate 5 minutes at room temperature.
2. Transfer mixtures to appropriate cuvettes and measure absorbance at 620 nm (A_{620}).
   Note: If A_{620} of a Sample is higher than the A_{620} for Standard 1, dilute Sample with ultrapure water and repeat the assay.

Results

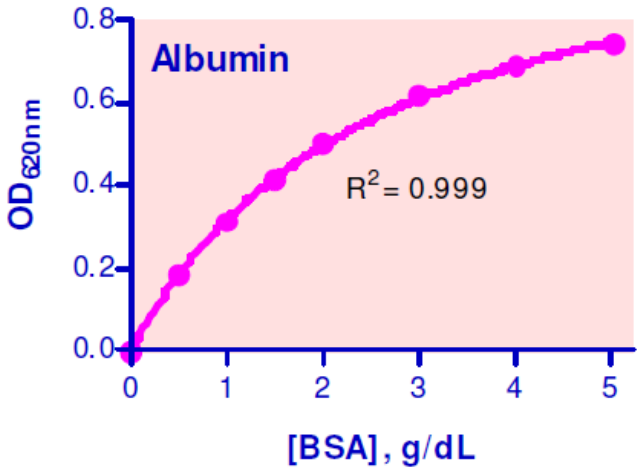
Calculations

Subtract the A_{620} of the Blank (0 g/dL) from the A_{620} of each Standard and plot the A_{620} against standard concentrations. Use the standard curve to determine the sample albumin concentration.

Conversion factors for albumin:
0.1 g/dL = 15 µM = 0.1% = 1,000 ppm

Typical Data

Standard curve is for demonstration only. A standard curve must be run with each set of assays.



Standard Curve in 96-well plate assay

Product Profile

Detection range (96 well plate assay):
1.5 µM (0.01 g/dL) to 750 µM (5 g/dL) albumin

Specificity

Albumin was assayed in duplicate using the 96 well assay procedure. The albumin content (g/dL) was 4.8±0.0 and 5.4±0.0 in human serum and plasma, 2.2±0.0 and 2.8±0.2 in rat serum and plasma, 3.2±0.2 in goat serum; and 2.0±0.0 in fetal bovine serum, respectively. Albumin in a fresh healthy human urine sample was below the detection limit (0.01 g/dL).

References

1. Lee, R.H. et al., Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD_scid mice. PNAS, **103(46)**, 17438–17443 (2006).
2. Rebecca, R. Associations of histories of depression and PMDD diagnosis with allopregnanolone concentrations following the oral administration of micronized progesterone Psychoneuro-endocrinology, **31(10)**, 1208-1219 (2006).
3. Cosgrove, D. et al., Integrin alpha1β1 Regulates Matrix Metalloproteinases via P38 Mitogen-Activated Protein Kinase in Mesangial Cells. Implications for Alport Syndrome. Am. J. Pathology, **172**, 761-773 (2008).

Troubleshooting Guide

Problem	**Possible Cause**	**Suggested Solution**
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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