GENTAUR

# DATA SHEET

## BCG Albumin Assay Kit

**Quantitative Colorimetric Albumin Determination at 620nm**

### DESCRIPTION

Albumin is the most abundant plasma protein in human. It accounts for about 60% of the total serum protein. Albumin plays important physiological roles, including maintenance of colloid osmotic pressure, binding of key substances such as long-chain fatty acids, bile acids, bilirubin, haematin, calcium and magnesium. It has anti-oxidant and anticoagulant effects, and also acts as a carrier for nutritional factors and drugs, as 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.

Simple, direct and automation-ready procedures for measuring albumin concentration in biological samples are becoming popular in Research and Drug Discovery. BCG albumin assay kit is designed to measure albumin directly in biological samples without any pretreatment. The improved method utilizes bromcresol green that forms a colored complex specifically with albumin. The intensity of the color, measured at 620nm, is directly proportional to the albumin concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

### KEY FEATURES

**Sensitive and accurate**. Use as little as 5 uL samples. Detection range 0.01 g/dL (1.5uM) to 5 g/dL (750uM) albumin in 96-well plate assay.

**Simple and high-throughput**. The procedure involves addition of a single working reagent and incubation for 5 min. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.

**Improved reagent stability and versatility**. The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.

**No interference in biological samples**. No pretreatments are needed. Assays can be directly performed on raw biological samples i.e., in the presence of lipid and protein.

### APPLICATIONS:

**Direct assays:** albumin in serum, plasma, urine, biological preparations.

**Drug discovery/Pharmacology:** effects of drugs on albumin metabolism.

### KIT CONTENTS (250 tests in 96-well plates)

**Reagent: 50 mL** **Albumin Standard: 1 mL 5 g/dL BSA**

**Storage conditions**. Store Reagent in the provided amber bottle and standard at 4°C and -20°C, respectively. Shelf life: 12 months.

**Precautions**: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

### PROCEDURES

### Reagent Preparation:

Important: bring reagent to room temperature and shake before use.

### Procedure using 96-well plate:

1. Dilute standards in distilled water as follows. Dilute serum and plasma samples 2 fold. Transfer 5 uL diluted standards and diluted samples to wells of a clear bottom plate. Store diluted standards at -20°C for future use.

| No | STD + $H_2O$ | Vol (uL) | BSA (g/dL) |
|---|---|---|---|
| 1 | 100uL + 0uL | 100 | 5.0 |
| 2 | 80uL + 20uL | 100 | 4.0 |
| 3 | 60uL + 40uL | 100 | 3.0 |
| 4 | 40uL + 60uL | 100 | 2.0 |
| 5 | 30uL + 70uL | 100 | 1.5 |
| 6 | 20uL + 80uL | 100 | 1.0 |
| 7 | 10uL + 90uL | 100 | 0.5 |
| 8 | 0uL + 100uL | 100 | 0 |

2. Add 200 uL working reagent and tap lightly to mix. Avoid bubble.

3. Incubate 5 min at room temperature and read optical density at 570-670nm (peak absorbance at 620nm). Signal is stable for > 60min.

### Procedure using cuvette:

1. Transfer 20 uL Blank, Standards and samples to appropriately labeled tubes. Add 1000 uL working reagent and tap lightly to mix. Incubate 5 min at room temperature.
2. Transfer to cuvet and read optical density at 620nm.

**Important:** if sample OD is higher than the OD for standard, dilute samples with distilled water and repeat the assay.

### CALCULATION

Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard concentrations. Use the standard curve to determine the sample albumin concentration, or fit the standard curve using the equation $y = a \cdot x / (b+x)$. The albumin concentration of Sample is calculated as

$OD_{SAMPLE} = (OD_{SAMPLE} - OD_{BLANK})$. *n* is the dilution factor.

**Conversions**: 0.1 g/dL albumin equals 15 uM, 0.1% or 1000 ppm.

### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. 5 uL).

### Procedure using 96-well plate:

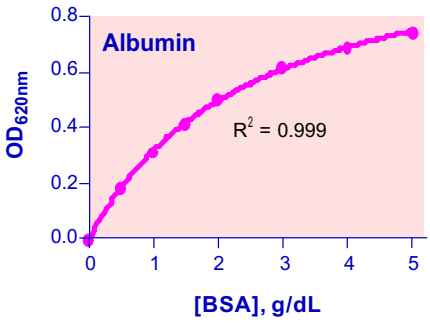
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

### Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD at 620nm.

### EXAMPLES:

Albumin was assayed in duplicate using the 96-well assay protocol. 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).




**Calibration curve** in 96-well plate
$y = 1.125 \cdot x / (2.535 + x)$

### LITERATURE


1. Nicholson, JP, Wolmarans, MR and Park, GR (2000). The role of albumin in critical ilness. Br. J. Anaesthesia 85(4): 599-610.

2. Goldwasser, P and Feldman, J (1997). Association of serum albumin and mortality risk. J. Clin. Epidemiol 50: 693-703.

3. Kamphuis, JS, Salden, HJM and Zuijderhoudt, FMJ (2001). Albumine-analyse in plasma: vergelijking tussen de brooncresol-groen, broomcresolpurper en een immunoassay bij volwassen patienten met en zonder hemodialyse. Ned Tijdschr Klin Chem 26: 9-12.



Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium