

## User's Manual and Instructions

### BCP Albumin Assay Kit (Z5030019)

#### Quantitative Colorimetric Albumin Determination at 610nm

#### 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, 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. Biochain's BCP albumin assay kit is designed to measure albumin directly in biological samples without any pretreatment. The improved method utilizes bromocresol purple that forms a colored complex specifically with albumin. The intensity of the color, measured at 610nm, 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 20  $\mu$ L samples. Detection range 0.3 g/dL (45 $\mu$ M) to 5 g/dL (750 $\mu$ M) 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 for thousands of samples per day.

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

**Low 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, 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:** 2 mL 5 g/dL BSA

**Storage conditions.** The kit is shipped at room temperature. Store Reagent and standard at 4°C and -20°C, respectively. Shelf life: 12 months after receipt.

**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 reagents to room temperature and shake before use.

##### Procedure using 96-well plate:

*Note: Bromocresol purple reacts differently with albumins from different species. Use albumin from the species of the samples being tested as the standard. For example for human samples, use HSA (Sigma Cat. No. A9511). If not measuring BSA, Prepare a 5 g/dL stock of the appropriate albumin in dH<sub>2</sub>O.*

1. Dilute standards in distilled water as follows.

No	STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Albumin (g/dL)
1	100 $\mu$ L + 0 $\mu$ L	100	5.0
2	80 $\mu$ L + 20 $\mu$ L	100	4.0
3	60 $\mu$ L + 40 $\mu$ L	100	3.0
4	40 $\mu$ L + 60 $\mu$ L	100	2.0
5	30 $\mu$ L + 70 $\mu$ L	100	1.5
6	20 $\mu$ L + 80 $\mu$ L	100	1.0
7	10 $\mu$ L + 90 $\mu$ L	100	0.5
8	0 $\mu$ L + 100 $\mu$ L	100	0

Dilute serum samples 2 fold. Transfer 20  $\mu$ L diluted standards and diluted samples to wells of a clear bottom plate. Store diluted standards at -20°C for future use.

2. Add 200  $\mu$ L working reagent and tap lightly to mix. Avoid bubble formation!
3. Incubate 5 min at room temperature and read optical density at 590-630nm (peak absorbance at 610nm).

#### Procedure using cuvette:

1. Transfer 60  $\mu$ L Blank, Standards and samples to appropriately labeled tubes. Add 1000  $\mu$ L working reagent and tap lightly to mix. Incubate 5 min at room temperature.
2. Transfer to cuvette and read optical density at 610nm.

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

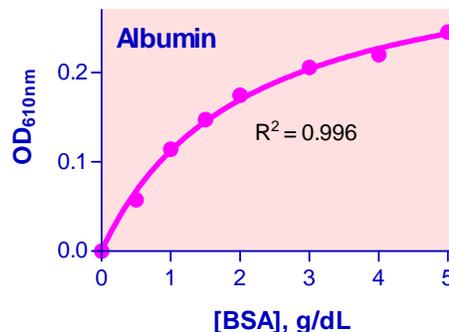
#### CALCULATION

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.

**Conversions:** 0.1 g/dL albumin equals 15  $\mu$ M, 0.1% or 1000 ppm.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Albumin standards other than BSA (e.g. human (Sigma cat # A9511), mouse (Sigma cat # A3139), rat (Sigma cat # A6414)). Pipetting devices and accessories, clear flat bottom 96-well plates (e.g. VWR cat # 82050-760) and plate reader or spectrophotometer and cuvettes for measuring OD at 610 nm.



Standard Curve in 96-well plate assay

#### PUBLICATIONS

1. Maier SM et al (2007) Proteinuria of Nonautoimmune Origin in Wild-type FVB/NJ Mice. *Comp Med.* 57(3):255-66.
2. Sharifuzzaman SM and Austin B (2009). Kocuria SM1 controls vibriosis in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *J Appl Microbiol.* 108(6): 2162 – 2170.
3. Shin SY et al (2009). Immunological investigation in the adenoid tissues from children with chronic rhinosinusitis. *Otolaryngol Head Neck Surg.* 141(1):91-6.