

QuantiChrom™ BCP Albumin Assay Kit (DIAP-250)

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. BioAssay Systems' 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 µL samples. Detection range 0.3 g/dL (45µM) to 5 g/dL (750µM) 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. Cuvet 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 x 1 mL 5 g/dL BSA

Storage conditions. Store Reagent 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 reagents to room temperature and shake before use.

Procedure using 96-well plate:

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

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

2. Add 200 µL 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). Signal is stable for > 60 min.

Procedure using cuvette:

1. Transfer 60 µL Blank, Standards and samples to appropriately labeled tubes. Add 1000 µL working reagent and tap lightly to mix. Incubate 5 min at room temperature.
2. Transfer to cuvet 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, or fit the standard curve using the equation $y = a \cdot x / (b + x)$. The albumin concentration of Sample is calculated as

$$= \frac{\text{AOD}_{\text{SAMPLE}} \times b}{a - \text{AOD}_{\text{SAMPLE}}} \times n \quad (\text{mg/dL})$$

$\text{AOD}_{\text{SAMPLE}} = (\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}})$. n is the dilution factor.

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

MATERIALS REQUIRED, BUT NOT PROVIDED

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

Procedure using 96-well plate:

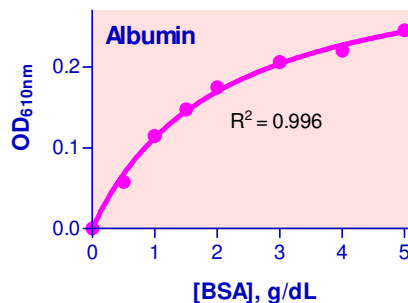
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and cuvetts for measuring OD at 610nm.

EXAMPLES:

Albumin was assayed in duplicate using the 96-well assay protocol. The albumin content (g/dL) was 3.6 ± 0.0 , 2.8 ± 0.1 and 4.3 ± 0.0 in rat serum, fetal bovine serum and goat serum, respectively. The albumin content in a fresh human urine sample was below the detection limit (0.3 g/dL).



Calibration curve in 96-well plate
 $y = 0.3433 \cdot x / (2.05 + x)$

LITERATURE

1. Nicholson, JP, Wolmarans, MR and Park, GR (2000). The role of albumin in critical illness. 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 broomcresol-groen, broomcresolpurper en een immunoassay bij volwassen patiënten met en zonder hemodialyse. Ned Tijdschr Klin Chem 26: 9-12.