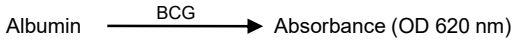
Albumin (BCG) Assay Kit (Colorimetric) (BN00784)

(Catalog #BN00784; 100 assays; Store at -20°C)

I. Introduction:

Albumin is the most abundant protein in human blood and is highly conserved among vertebrates. It plays a pivotal physiological role in maintenance of plasma osmotic pressure, vascular permeability, and transport of cholesterol, bile pigments, nitric oxide, metals, and other small molecules in the body. It also functions as a free radical scavenger of reactive oxygen and nitrogen species, triggers cell signaling processes, possesses anti-inflammatory and coagulatory effects. Assay Genie's Albumin (BCG) Assay Kit is a simple high-throughput assay that detects Albumin concentration in serum. The assay is based on the selective interaction between Bromocresol Green (BCG) and Albumin forming a chromophore that can be detected at 620 nm. The signal is directly proportional to the amount of Albumin present in the serum. BCG does not react with other abundant plasma proteins like IgG. The assay can detect as low as 5 µg (0.01 g/dl) of albumin in serum samples.



II. Application:

- Measurement of Albumin concentration in serum

III. Sample Type:

- Biological fluids: Serum

IV. Kit Contents:

Components	BN00784	Cap Code	Part Number
Albumin Assay Buffer	25 ml	WM	BN00784-1
Bromocresol Green (BCG)	100 µl	Red	BN00784-2
Bovine Serum Albumin (BSA, 50 mg/ml)	0.5 ml	Yellow	BN00784-3

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read the entire protocol before performing the assay.

- **Albumin Assay Buffer**: Bring to room temperature (RT) before use. Store at -20°C.
- **Bromocresol Green (BCG):** Ready-to-use as supplied. Light sensitive. Warm to RT before use. Store at -20°C.
- **Bovine Serum Albumin (BSA):** Ready-to-use as supplied. Aliquot and Store at -20°C. Bring to RT before use.

VII. Albumin (BCG) Assay Protocol:

1. **Sample Preparation:** Add 2-50 µl of undiluted serum into desired well(s) in a 96-well plate. Adjust the volume to 50 µl/well with Albumin Assay Buffer.

   Notes:

   a. For unknown samples, we recommend doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.

   b. Albumin concentration is over a wide range depending on the sample and species, for example, in healthy humans it is between 3.5-5 g/dl. Patients with hypoalbuminemia and hyperalbuminemia shows albumin levels less than 3.5 g/dl and greater than 5 g/dl, respectively.

   c. Optional: If sample has intrinsic high absorbance at 620 nm, prepare parallel sample well(s) as sample background control(s) and adjust the volume to 50 µl/well with Albumin Assay Buffer.

2. **Standard Curve Preparation:** Add 0, 2, 4, 6, 8, and 10 µl of 50 mg/ml BSA Standard into a series of wells in a 96-well plate to generate 0, 100, 200, 300, 400 and 500 µg of Albumin/well. Adjust the volume to 50 µl/well with Albumin Assay Buffer.

   Notes:

   a. Always prepare fresh Standard Curve solutions and use within 24 hrs.

   b. Optional: For a more sensitive assay (linear range), prepare 7.5 mg/ml BSA Standard by adding 15 µl of 50 mg/ml Standard into 85 µl ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 7.5 mg/ml BSA Standard into a series of wells in a 96-well plate to generate 0, 15, 30, 45, 60, and 75 µg of Albumin/well.

3. **Reagent Mix:** Dilute Bromocresol Green (BCG) stock solution 1:10 by adding 10 µl of stock solution to 90 µl of ddH_2O as needed. Mix enough reagents for the total number of well(s) to be assayed including Standards and samples.

	Reagent Mix	*Background Control
Albumin Assay Buffer	96 µl	100 µl
Diluted Bromocresol Green (BCG)	4 µl	—

Add 100 µl of Reagent Mix to each well. Mix well.

* Add 100 µl of Background Control mix to sample background control well(s). Mix well.

4. **Measurement:** Incubate plate at RT (~25°C) for 20 min., protected from light. Measure absorbance at 620 nm in a plate reader.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Albumin Standard Curve. If sample background control is significant, then subtract sample background control reading from sample reading. Apply corrected OD to the BSA Standard Curve to get B µg of Albumin in the sample well.

Sample Albumin Concentration (C) = B/V X D µg/µl

Where: **B** is the amount of Albumin in the sample well (µg)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

BSA molecular weight ≈ 66.5 kDa
10 mg/ml ≡ 10 µg/µl ≡ 10000 µg/ml ≡ 1 g/dl

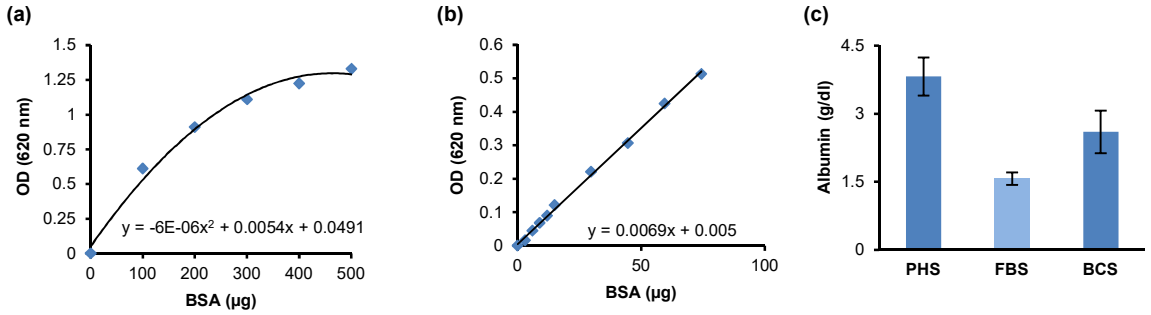


Figure: (a) BSA Standard Curve (0-500 µg). **(b)** BSA Standard Curve (0-75 µg). **(c)** Albumin concentration in pooled human serum (PHS), fetal bovine serum (FBS) and bovine calf serum (BCS). Sample volumes (0-20 µl) were assayed following the kit protocol. Albumin concentration (g/dl): PHS: 3.8 ± 0.4; FBS: 1.6 ± 0.1; BCS: 2.6 ± 0.5.

FOR RESEARCH USE ONLY! Not to be used on humans.