

# Albumin Kit Instructions

For the quantitative determination of albumin in human serum or plasma

Catalog #80389 100 Assays

For research use only. Not for use in diagnostic procedures.

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# TABLE OF CONTENTS

A.	Intended Use	1	
B.	Introduction	1	
C.	Principles of the Assay	1	
D.	Kit Storage	1	
E.	Assay Materials  E.1. Materials provided  E.2. Materials to be supplied by user		
F.	Assay Precautions & Limitations	2	
G	Maximizing Kit Performance	2	
Н.	Sample Collection	2	
I.	Assay Procedure		
	I.1. Preparation of reagents	2	
	I.2. Preparation of samples, calibrators, and controls	2	
	I.3. Assay procedure – Total Albumin (g/dL)	3	
	I.4. Determining the Total Albumin (g/dL) concentration	3	
J.	Performance characteristics		
	J.1. Assay range	3	
	J.2. Precision	3	
Wa	Warranty		

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#### A. Intended Use

The Albumin kit is for the quantitative determination of albumin in human serum or plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

#### B. Introduction

Albumin is the most abundant of the serum proteins. Its importance in body function is its ability to transport substances such as drugs, antibiotics, bilirubin and fatty acids. Albumin also helps maintain body osmotic pressure and serves as a storehouse of structural proteins that are used for tissue growth. High albumin levels usually reflect dehydration. Lowered albumin levels occur commonly in a variety of diseases such as nephritic syndrome, liver diseases, acute infections and malnutrition, making albumin determinations of prime significance.

# C. Principle of the Assay

Human albumin concentration is determined based on the dye-binding properties of serum albumin with bromocresol green (BCG). The absorbance of BCG at 600 nm increases with binding to albumin, and is proportional to the albumin concentration present.

# D. Kit Storage

- 1. Upon receipt of the Albumin kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
- 2. The kit should not be used after the expiration date.

#### E. Assay Materials

#### E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
TA1	Reagent Total Albumin 1 (liquid)	1 X 25 mL
TACAL1	Total Albumin Calibrator 1 (lyophilized)	1 X 5 mL

Note: Optional controls are available separately (Cat# 80383)

#### E.2. Materials required but not provided

Microplates

Micropipettes and disposable tips

Clean glass tubes and test tube racks

Volumetric flasks

Incubator (37°C)

Distilled water

0.9% saline

Microplate reader or spectrophotometer or chemistry analyzer (should read  $A_{600}$  values)

# F. Assay Precautions & Limitations

- Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
- 2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
- 3. The binding of BCG to human albumin is not wholly specific as some dye binds to a2 and b2 globulins although typically slower than albumin.
- 4. Do not use the reagents after the expiration date.

# G. Maximizing Kit Performance

- 1. Given the small sample volumes required (2  $\mu$ L), pipetting should be done as carefully as possible. A high quality 10  $\mu$ L or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
- 2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
- 3. Each calibrator and sample should be assayed in duplicate.
- 4. The same sequence of pipetting and other operations should be maintained in all procedures.
- 5. Do not mix reagents that have different lot numbers.

# H. Sample Collection

**Serum**: Collect blood, allow to clot, and centrifuge for 20 min at 2,000 x g.

**Plasma**: Collect blood into a tube containing EDTA (final concentration: 0.1%), and centrifuge for 20 min at 2000 x *g*.

It is recommended that samples be used within 2 weeks of collection when stored refrigerated. If assay is to be performed more than 2 weeks after collection, samples should be frozen.

# I. Assay Procedure

#### I.1. Preparation of reagents

All reagents are provided ready-to-use and should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

#### I.2. Preparation of samples, calibrators, and controls

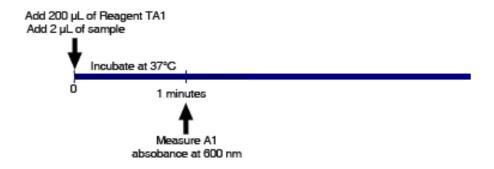
- 1. The Total Albumin Calibrator 1 (marked as "TACAL1") is provided in lyophilized form and must be reconstituted with 5.0 mL of distilled water. To ensure complete reconstitution, equilibrate vial at room temperature for 30 minutes before first use.
  - Note: Reconstituted calibrators are stable for 14 days when capped tightly and stored at 2-8°C. In addition to running the calibrator provided, the assay requires running a blank calibrator. 0.9% saline should be used for running the blank calibrator. Optional controls are sold separately (Cat# 80333).
- 2. Bring all samples, calibrators, and controls to room temperature. Frozen samples should be allowed to fully thaw before proceeding.
- 3. Prior to testing, serum samples, calibrators, and controls should be thoroughly mixed by gentle inversion.

# I.3. Total albumin (g/dL) assay procedure

The procedure below reflects a manual procedure performed using a microplate and a microplate reader (ideal when running multiple samples). The procedure can be easily adopted as needed to be run in a glass tube with a spectrophotometer. The assay can also be adopted to work on various automated analyzers. Please contact Crystal Chem for more information.

- 1. Add 200 µL of Reagent TA1 into each well (as needed) of a microplate and mix well by repeated pipetting.
- 2. In each well, add 2 μL of sample, calibrator, or control and mix well by repeated pipetting.
- 3. Place microplate in incubator (37°C) and allow microplate to equilibrate to 37°C over 1 minute.
- 4. Measure absorbance using a plate reader (measure A<sub>600</sub> values).

Figure 2. Summary of assay procedure



# I.4. Determining the Total Albumin (g/dL) concentration

1. Using linear graph paper, construct the total albumin calibration curve by plotting the mean absorbance value for each calibrator (including the zero calibrator) on the Y axis versus the corresponding calibrator total albumin concentration on the X axis.

**Note:** Calibrator values vary per lot and should be obtained from the calibrator label. A calibration curve should be plotted every time the assay is performed.

2. Total albumin concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The total albumin concentration is expressed in g/dL.

**Note:** Samples with high total albumin concentrations (6.0 g/dL or higher) should be diluted and rerun.

# J. Performance characteristics

#### J.1. Assay range

The Albumin assay has a linear range from 0 – 6 g/dL.

#### J.2. Precision

The assay has a within-run and total precision of CV < 10%.

# Catalog #80389

#### Warranty

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