Albumin (BCG) Colorimetric Assay Kit

Catalog No. KM0012

Detection and Quantification of Albumin Concentrations in Biological Samples.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.
INTRODUCTION

Albumin is a term for any protein that is water soluble and can be denatured with heat. It is usually found in blood plasma and unlike most blood proteins, they are not glycosylated. It regulates the colloidal osmotic blood pressure and is the main protein of plasma. Substances that contain albumin are commonly known as albuminoids.

In this assay, Albumin reacts with bromocresol green to produce a colored complex with absorbance maxima at approximately 620 nm.

FEATURES

**Number of Assays:** 100 Assays  
**Samples:** serum, plasma, urine, etc.  
**Note:** This assay kit is validated with serum.  
**Colorimetric Dynamic Range:** 6 to 50 mg/ml Albumin  
Absorbance at 620 nm

STORAGE & HANDLING

Upon receipt, take out the Albumin Standard and store at -20°C. Store all other components in the original box at room temperature. The kit should be stable for at least six months.

**Note:** Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
KIT CONTENTS & STOCK PREPARATION

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Storage</th>
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<tbody>
<tr>
<td>96-Well Microplate</td>
<td>1 plate</td>
<td>RT</td>
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<tr>
<td>Reaction Buffer</td>
<td>25 ml</td>
<td>RT</td>
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<tr>
<td>Albumin Standard (Blue Cap)</td>
<td>1 ml of 500 mg/ml</td>
<td>-20°C</td>
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<tr>
<td>Dye Reagent (Green Cap)</td>
<td>21 ml</td>
<td>RT</td>
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Note: If not running entire plate at once, aliquot and store reagents under conditions according to this table. Use within 2 months.

MATERIALS NOT PROVIDED

Pipetting devices, tubes, and microplate reader.

ASSAY RESTRICTIONS

- This kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in performance of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.
REAGENT & SAMPLE PREPARATIONS
Please read the manual in its entirety before proceeding with the assay.

Note: Warm all reagents to room temperature before use and protect from light. Calculate and prepare the amount needed if a full plate is not used.

1. Preparation of Standard
The Albumin Standard is provided at a 500 mg/ml stock concentration.

To prepare the standard, dilute the appropriate amount of Albumin stock solution in Reaction Buffer to create 7 concentrations from a high point of 50mg/ml down to a low point of 6mg/ml for the standard. The last row consists of only Reaction Buffer to serve as a negative control. The standards are run in duplicate. Use a 96-well clear, flat bottom plate.

2. Preparation of Samples
Dilute the samples in Reaction Buffer. A variable dilution will be required depending on the total amount of Albumin present in your sample. In the first trial, the samples should be serially diluted to determine the optimal amount of sample for the assay. For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.
ASSAY PROTOCOL

1. Add 5µl of each sample/standard to each well in a 96-well clear, flat bottom plate. The standards are run in duplicate. It is advised to run the samples in duplicate or triplicate.

Sample Plate Layout (you do not have to follow this)

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Note: BL=Blank Control, ST=Albumin Standards, EX=Experimental Test Samples

2. Add 200 µl of the Dye Reagent to each well of the microplate containing the standards, controls, and samples.

3. Read the plate within 5 minutes using a microplate reader at 620 nm.
STANDARD CURVE

Correct for background absorbance. For each point, subtract the value derived from the negative control. Plot the Albumin concentrations vs. the OD values to create the standard curve.

![Standard Curve Graph]

Albumin (BCG) Colorimetric Assay Kit allows for the detection and quantification of endogenous Albumin concentrations within the range of 6.25 mg/ml - 50 mg/ml in cell lysates, sera, and plasma.

CALCULATION

Determine the slope of the standard curve and calculate the Albumin concentration of your samples.

\[
[\text{Albumin}] = \frac{(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}})}{\text{Slope}} \times \text{Dilution Factor}
\]

Conversions: 50 mg/mlAlbumin = 5000 mg/dl = 5 % = 50000 ppm
TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com or contact us at tech@immunoway.com

NOTES