Albumin Assay Kit

Catalog No.: abx298891

Size: 96 tests

Detection Range: 0.08 g/L – 15 g/L

Sensitivity: 0.08 g/L

Storage: Store the Chromogenic Reagent at 4°C in the dark. Store the Standards at -20°C.

Application: For detection and quantification of Albumin concentration in serum, plasma, and cell culture supernatants.

Introduction

Abbexa's Albumin Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Albumin concentration. Bromocresol Green dye combines with Albumin at pH 4.0 – 4.2, forming a blue-green colored complex with an absorbance maximum at 630 nm. The intensity of the color is proportional to the Albumin concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Chromogenic Reagent: 6 ml
3. Standard (20 g/L): 2 × 1.2 ml
4. Plate sealer: 2

Materials required but not provided

1. Microplate reader (630 nm)
2. Double-distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Chromogenic Reagent Working Solution:** Prepare enough Chromogenic Reagent Working Solution for the wells tested. Mix the Chromogenic Reagent and double-distilled water in a ratio of 1 : 4. Prepare 250 µl Chromogenic Reagent Working Solution per well by mixing 50 µl Chromogenic Reagent with 200 µl double-distilled water. Prepare immediately before use.
- **Standards:** Standard vials should be taken from storage and placed on ice to thaw gradually. Label 7 tubes with 1.0 g/L, 2.0 g/L, 3.5 g/L, 5.0 g/L, 8.0 g/L, 12.0 g/L, and 15.0 g/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (g/L)	**1.0**	**2.0**	**3.5**	**5.0**	**8.0**	**12.0**	**15.0**
20 g/L Standard (µl)	10	20	35	50	80	120	150
Double distilled water (µl)	190	180	165	150	120	80	50

For the blank, use pure double-distilled water. The volume of each standard dilution will be 200 µl.

Note:

- Allow all Chromogenic Reagent to equilibrate to room temperature before use.
- Keep Standard on ice during use, avoid repeated freeze-thaw cycles.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -80°C for up to one month. Avoid multiple freeze-thaw cycles.

- **Serum/Plasma:** Samples can be tested directly.
- **Cell Culture Supernatant:** Samples can be tested directly.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	**Dilution Factor**
Human serum	8 – 15
Human plasma	8 – 15
HepG2 supernatant	1
Mouse plasma	8 – 15
Rat serum	8 – 15

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

B. Assay Procedure

1. Mark positions on the 96-well microplate for each standard, blank, and sample. *It is strongly recommended to test all wells in duplicate. Avoid foaming or bubbles.*
2. Add 10 µl of each standard dilution to the corresponding standard wells.
3. Add 10 µl of sample each sample well.
4. Add 250 µl Chromogenic Reagent Working Solution to each well.
5. Incubate for 10 minutes at room temperature.
6. Measure the OD of each well with a microplate reader at 630 nm.

C. Calculation of Results

Plot the standard curve, using the mean OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$.

Based on this curve, the concentration of Albumin in each sample well can be derived using the following formula:

1. **Serum/Plasma/Cell Culture Supernatant samples:**

$$\textbf{Albumin Concentration (g/L)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}} - \mathbf{b})}{\mathbf{a}} \times \mathbf{F}$$

where:

OD_{Sample}	OD of the sample wells
OD_{Blank}	OD of the blank wells
a	Gradient of the standard curve ($y = \mathbf{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \mathbf{b}$)
F	Dilution factor of the sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.