MOUSE ALBUMIN ELISA
Life Diagnostics, Inc., Catalog Number: ALB-1

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INTRODUCTION

Albumin is the most abundant protein found in serum (~ 40 mg/ml). It has a molecular weight of ~65,000 and plays a vital role in regulating the intravascular colloid osmotic pressure. It also serves as a carrier protein for steroid hormones and fatty acids. It is classified as a negative acute phase reactant because its serum levels can decrease by approximately 30% in response to disease, tissue injury, or inflammation.[1-3]

PRINCIPLE OF THE TEST

The mouse albumin ELISA uses affinity purified anti-albumin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-albumin antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. This results in albumin molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of albumin is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-mouse albumin antibody coated 96-well microtiter plate (provided as 12 detachable strips of 8)
- Enzyme conjugate reagent, 11 ml
- Reference standard (lyophilized)
- 10x Diluent, 50 ml
- 20x Wash solution, 50 ml
- TMB reagent (One-Step), 11 ml
- Stop solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE

The unused kit should be stored at 2-8°C, and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18- 25^{o}C) before use.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The mouse albumin standard is provided as a lyophilized stock. Add the volume of distilled or deionized water indicated on the vial label and mix gently until dissolved ***(the reconstituted standard remains stable for at least one week at 2-8^{o}C, but should be aliquoted and frozen at -20^{o}C if use beyond this time is intended)***.
2. Label 7 polypropylene or glass tubes as 200, 100, 50, 25, 12.5, 6.25, and 3.13 ng/ml.
3. Into the tube labeled 200 ng/ml, pipette the volume of diluent detailed on the reference standard vial label. Then add the indicated volume of reconstituted standard and mix gently. This provides the 200 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 100, 50, 25, 12.5, 6.25, and 3.13 ng/ml.
5. Pipette 250 µl of the 200 ng/ml albumin standard into the tube labeled 100 ng/ml and mix. This provides the working 100 ng/ml standard. Similarly prepare the 50, 25, 12.5, 6.25, and 3.13 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Serum levels of albumin are ~ 40 mg/ml. Serum and plasma samples must therefore be significantly diluted prior to assay. We suggest an initial dilution of 800,000. This is best achieved by serial dilution:

1. Dispense 995 µl of 1 x diluent into two tubes and 475 µl into a third tube for each sample to be tested.
2. Dilute 5 µl of sample into one of the tubes containing 995 µl of diluent and mix gently. This provides a 200-fold diluted sample.
3. Dilute 5 µl of the 200-fold diluted sample into the second tube containing 995 µl of diluent and mix gently. This provides a 40,000-fold diluted sample.
4. Dilute 25 µl of the 40,000-fold diluted sample into the tube containing 475 µl of diluent and mix gently. This provides an 800,000-fold diluted sample.

Repeat the above procedure for each sample.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.

4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
8. Wash as detailed in steps 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

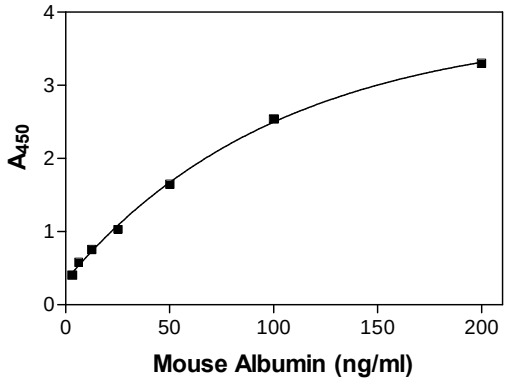
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of albumin in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of albumin in the serum sample.
5. If available, PC graphing software may be used for the above steps.
6. If the A_{450} values of samples fall outside the standard curve samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against albumin concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

Albumin (ng/ml)	A_{450}
200	3.091
100	2.381
50	1.532
25	1.027
12.5	0.634
6.25	0.473
3.13	0.389



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

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2. Eberini I, et al. Proteins of rat serum IV. Time-course of acute-phase protein expression and its modulation by indomethacine. Electrophoresis 20: 846-853 (1999)
3. Whalen R, Voss SH and Boyer TD. Decreased expression levels of rat liver glutathione S-transferase A2 and albumin during the acute phase response are mediated by HNF1 (hepatic nuclear factor 1) and IL6DEX-NP. Biochem J. 377:763-768 (2004)

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