Mouse Albumin ELISA Kit

Catalog No. IMSALBKT SAMPLE

This mouse albumin antigen assay is intended for the quantitative determination of total albumin in mouse plasma, serum, urine & other biological fluids

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Background

Albumin is a water-soluble protein with considerable structural stability which makes up 60% of the total protein of plasma. It functions as a carrier of hormones, enzymes, fatty acids, metal ions, and medicinal products.

Assay Principle

Mouse albumin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, peroxidase labeled polyclonal anti-mouse albumin antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of mouse albumin. Color development is proportional to the concentration of albumin in the samples.

Reagents Provided

96-well microtiter strip plate

8X12 well removable strips containing affinity purified anti-mouse albumin antibody dried and blocked on the surface.

5X Diluent

1 bottle of 50mL; bring to 1X using DI water

10X Wash Buffer

1 bottle of 50mL; bring to 1X using DI water

Mouse albumin standard

1 vial of lyophilized standard

Peroxidase conjugated anti-mouse albumin primary antibody

1 vial of concentrated HRP-labeled antibody

TMB substrate solution

1 bottle of 10mL solution

Storage and Stability

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. DO NOT freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Required Reagents and Equipment

1-channel pipettes covering 0-10µL and 200-1000µL
12-channel pipette covering 30-300µL
Paper towels or kimwipes
50mL tubes, 1.5mL centrifuge tubes
1N H_2SO_4
DI water
Magnetic stirrer and stir-bars
Plastic containers with lids
Microtiter plate spectrophotometer operable at 450nm
Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warnings and Precautions

Warning – Avoid skin and eye contact when using TMB substrate solution. It may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

- DO NOT mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- DO NOT pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. DO NOT pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

Preperation of Reagents

Diluent concentrate: The diluent is supplied in a 5X concentrate and must be diluted 1:5 with deionized water for use with the kit.
Wash buffer concentrate: The wash buffer is supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

Sample Dilution

The assay measures total mouse albumin in the 1-1000 ng/ml range. Samples giving mouse albumin levels above 1000ng/ml should be diluted in 1X diluent before use. A 1:500,000 to 1:1,000,000 dilution for normal plasma, or a 1:1000 dilution for mouse urine is suggested for best results.

Assay Procedure

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:

Reconstitute standard vial with 1 ml of 1X diluent to give a 1000ng/ml solution.

Dilution table

Albumin concentration (ng/ml)	Dilutions
1000	*Straight from the vial*
500	*500µl (1X diluent) +500µl (1000ng/ml)*
200	*600µl (1X diluent) +400µl (500ng/ml)*
100	*500µl (1X diluent) +500µl (100ng/ml)*
50	*500µl (1X diluent) + 500µl (100ng/ml)*
20	*600µl (1X diluent) +400µl (50ng/ml)*
10	*500µl (1X diluent) +500µl (20ng/ml)*
5	*500µl (1X diluent) +500µl (10ng/ml)*
2	*600µl (1X diluent) +400µl (5ng/ml)*
1	*500µl (1X diluent) +500µl (2ng/ml)*
0	*500µl (1X diluent)*
	-Zero point to determine background

Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100µl standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:

Dilute 3µl of HRP conjugated primary antibody into 10ml of 1X diluent and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow.Mix thoroughly and read final absorbance values at 450nm. For best

Assay Procedure Continued

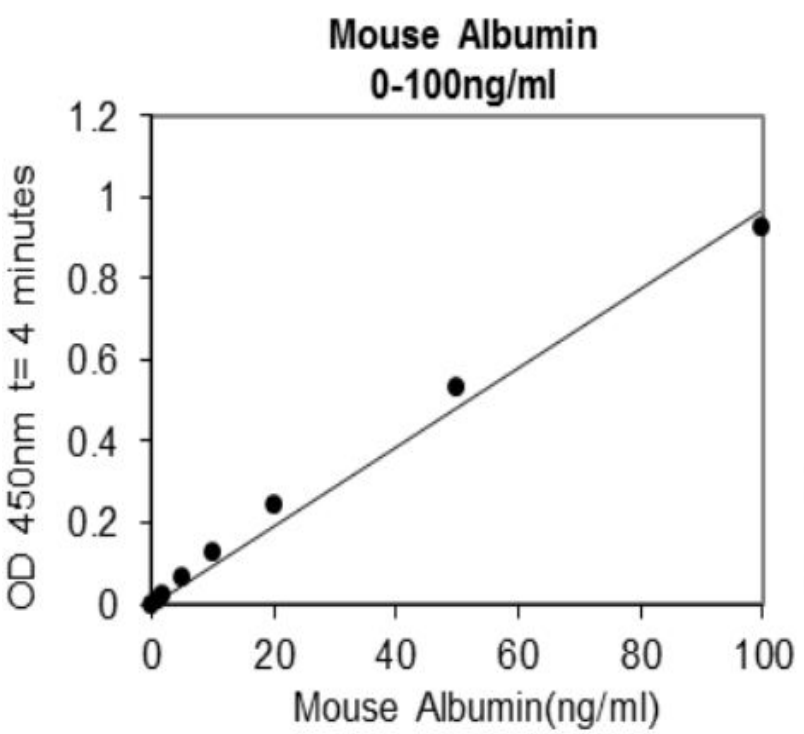
Measurement:

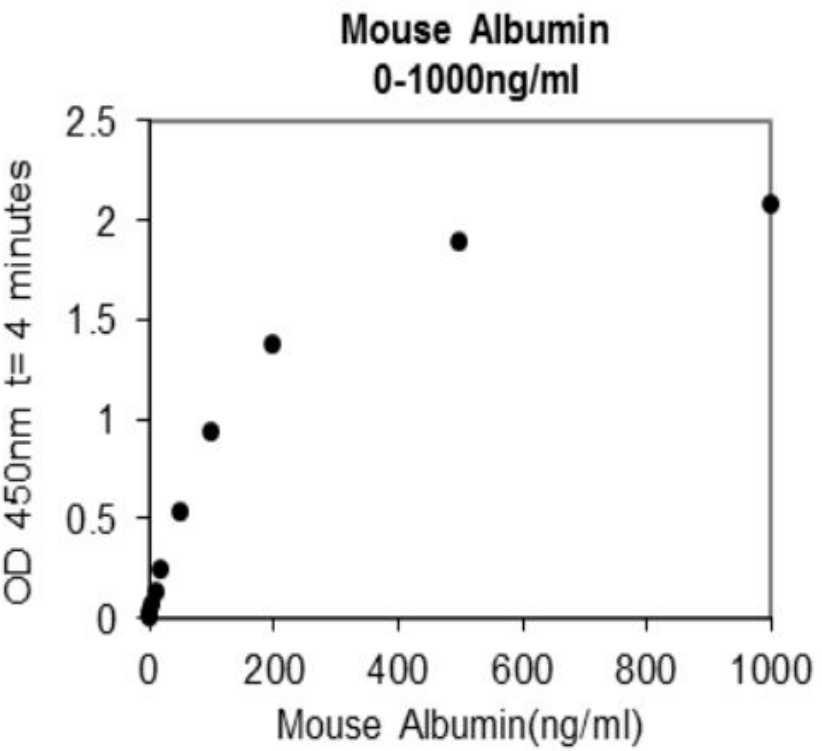
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).

Assay Calibration:

Plot A450 against the amount of mouse albumin in the standards. Fit a straight line through the linear points of the standard curve points using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of total mouse albumin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

Standard Curve Examples





Expected Values

Albumin is present in normal mouseserum at a concentration of 20 mg/ml in Balb/C, 27 mg/ml in C57BL6, and 29 mg/ml in CD1 strains [1].This kit has been validated for measurement of albumin in BALB/cmouse urine (33ug/ml, 1:1,000 dilution), Nude mouse urine (19ug/ml, 1:1,000 dilution), and CD1 mouse plasma (29mg/ml, 1:1,000,000 dilution).

Performance Characteristics

The average background OD of the assay is 0.050. The minimum detectable dose (MDD) of mouse albumin is 0.51 ng/ml. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero point replicates and calculating the corresponding concentration.

Disclaimer

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.