cellsciences.com

Mouse Albumin Antigen ELISA Kit

Catalog No: CS376A
CS376B

Size: 1 x 96 tests
5 x 96 tests

Sensitivity:	0.09 ng/ml
Specificity:	Mouse albumin (Does not distinguish IgG subclasses.)
Range:	0.2-200 ng/ml
Sample Type:	Mouse serum, plasma, urine and other biological fluids. **For research use only.**
Cross-Reactivity:	Pooled normal plasma from rat was assayed and slight cross-reactivity was observed. Pooled normal plasma from rabbit, dog, pig, sheep cynomolgus monkey and human was assayed and no significant cross-reactivity was observed.

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 450 nm. 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:

Description	Quantity
CS376A-P. 96-well microtiter strip plate coated with anti-Mouse albumin antibody, blocked and dried on well surface	1 plate: 96 wells (12 strips x 8 wells)
CS376A-A. 10X Wash Buffer Concentrate	1 bottle, 50 mL
CS376A-B. 5X Diluent	1 bottle, 50 mL
CS376A-C. Mouse albumin standard, lyophilized	1 vial
CS376A-D. Anti-Mouse albumin primary antibody, concentrated polyclonal Ab	1 vial
CS376A-E. TMB substrate solution	1 bottle, 10 ml

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

cellsciences.com

Mouse Albumin Antigen ELISA Kit

Storage and Stability:

All kit components must be stored at 2-8°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°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 2-8°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- Pipettes covering 0-10 µl and 200-1000 µl, and tips
- 12-channel pipette covering 30-300µl
- Paper towels or laboratory wipes
- Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
- 1N H_2SO_4 or 1N HCl
- Deionized or distilled water
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
- Automatic plate washer or wash bottle

Warnings:

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

Precautions:

- **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.

Preparation of Reagents:

- **1X Diluent:** 5X Diluent may contain precipitate. Warm to redissolve before use. Dilute 50 ml of 5X diluent concentrate with 200 ml of deionized water.
- **1X Wash buffer:** Dilute 50 ml of 10X wash buffer with 450 ml deionized water

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq$ - 20°C. Avoid repeated freeze-thaw cycles. Urine samples may be affected by freeze/thaw cycles or centrifugation.

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

cellsciences.com

Mouse Albumin Antigen ELISA Kit

Assay Procedure: Allow microtiter strips and assay components to warm to room temperature for 30 minutes. Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute Standard by adding **1.0 ml of diluent** directly to the vial and agitate gently to completely dissolve contents. This will result in a **1000 ng/ml** standard solution.

Table 1: Dilution table for preparation of Mouse Albumin standard:

IgG Concentration (ng/ml)	Dilutions
1000	Straight from the vial)
500	500 µl (Diluent) + 500 µl (1000ng/ml)
200	600 µl (Diluent) + 400 µl (500 ng/ml)
100	500 µl (Diluent) + 500 µl (200 ng/ml)
50	500 µl (Diluent) + 500 µl (100 ng/ml)
20	600 µl (Diluent) + 400 µl (50 ng/ml)
10	500 µl (Diluent) + 500 µl (20 ng/ml)
5	500 µl (Diluent) + 500 µl (10 ng/ml)
2	600 µl (Diluent) + 400 µl (5 ng/ml)
1	500 µl (Diluent) + 500 µl (2 ng/ml)
0	500 µl (Diluent) Zero point to determine background

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

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

NOTE: The assay measures total albumin antigen in the 1.0 - 1000 ng/ml range. If the unknown is thought to have higher albumin levels, dilutions may be made in diluent. A 1:1,000,000 to 1:4,000,000 dilution for normal mouse plasma or a 1:1,000 dilution for normal mouse urine is suggested for best results.

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

Mouse Albumin Antigen ELISA Kit

Primary Antibody Addition:
Briefly centrifuge vial before opening. Dilute **4 µl** of conjugated primary antibody in **10 ml Diluent**. Add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different intensities of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 or HCl 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 by gently shaking the plate and read plate immediately.

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

Assay Calibration:
Plot A_{450} against the amount of albumin in the standards. Fit a straight line through the linear points of the standard curve 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 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.

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

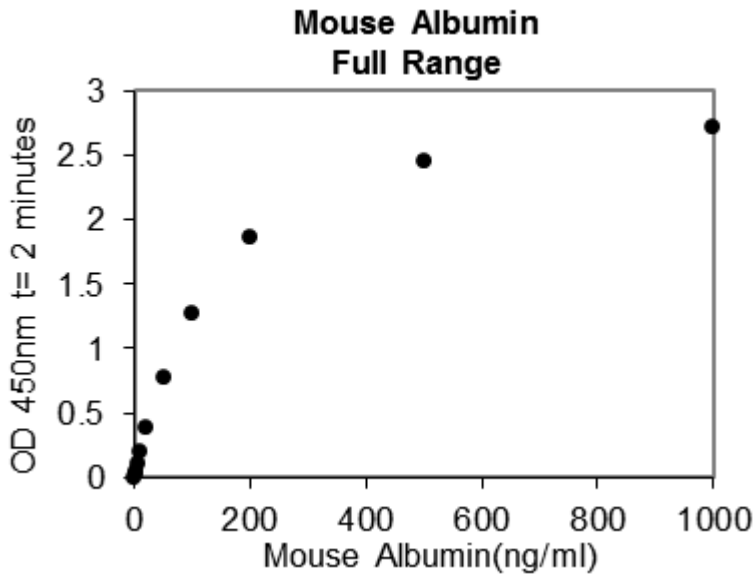
Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

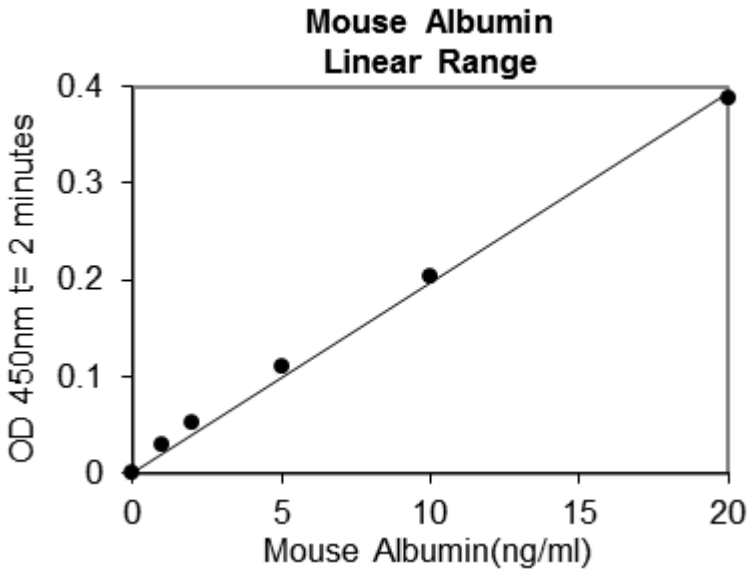
E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

cellsciences.com

Mouse Albumin Antigen ELISA Kit

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





Expected Values: Albumin is present in in normal mouse serum is 20 mg/ml in BALB/c, 27 mg/ml in C57BL6 and 29 mg/ml in CD-1 strains.

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

cellsciences.com

Mouse Albumin Antigen ELISA Kit

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.046-0.052) and calculating the corresponding concentration. The MDD was 0.09 ng/ml.

Specificity: Pooled normal plasma from rat was assayed and slight cross-reactivity was observed. Pooled normal plasma from rabbit, dog, pig, sheep, cyno monkey, and human was assayed and no significant cross-reactivity was observed.

Intra-assay Precision: 3 samples of known concentration were tested 20 times on 1 plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	20.6	81.3	185
Standard Deviation	1.30	3.26	11.1
CV (%)	6.31	4.01	5.98

Inter-assay Precision: These studies are currently in progress.

Recovery: These studies are currently in progress.

Linearity: To assess the linearity of the assay, samples of diluent spiked with high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	99	100	96	93
Range (%)	96-101	94-104	92-101	78-107

Sample Values: Samples were evaluated for the presence of antigen at varying dilutions.

Sample Type	Dilution	Mean (µg/ml)
CD-1 Citrate Plasma	1:1,000,000	29,000
BALB/c Urine	1:1,000	33
Nude Mouse Urine	1:1,000	19

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

cellsciences.com

Mouse Albumin Antigen ELISA Kit

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com