MOUSE SERUM ALBUMIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE ALBUMIN CONCENTRATIONS IN SERUM AND EDTA PLASMA

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	MOUSE SERUM ALBUMIN ELISA
Catalog No.	SK00383-03
Lot No.	
Formulation	96 T
Standard Range	1.56 -100 ng/mL
Sensitivity	0.3 ng/mL
Sample Require	5~ 10 µL
Dilution Factor	1,000,000~4,000,000 (1000K~ 4000K) (**Optimal dilutions should be determined by each laboratory for each application**)
Sample Type	Serum and EDTA Plasma
Specificity	Mouse Albumin
Calibration	Mouse Albumin
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8°C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Mouse Albumin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural mouse albumin from serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains mouse albumin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural mouse albumin samples.

ASSAY OVERVIEW

The Mouse Albumin ELISA kit is based on the binding of mouse albumin in samples to two antibodies. One has been pre-coated onto a microplate, and the other is conjugated to HRP. Standards and samples are pipetted into the wells and any albumin present is bound by the immobilized antibody. After a washing step, the antibody-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of albumin bound in the initial step. The color development is stopped and the intensity of the color is measured.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Albumin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against mouse albumin.	**383-03-01**	**1 plate**
Albumin Standard – 1200 ng/vial of mouse albumin for calibration in a buffered protein base with preservative; lyophilized.	**383-03-02**	**1 vial**
Detection Antibody-HRP Conjugate – 120 µL/vial, 100-fold concentrated antibody-HRP conjugate against mouse albumin.	**383-03-03**	**1 vial**
Positive Control – one vial of 10-fold concentrated mouse albumin; lyophilized.	**383-03-04**	**1 vial**
Dilution Buffer Concentrate – 60 mL of 5-fold concentrate buffered protein based solution with preservative.	**DB16F**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 1 month. For longer storage for up to 8 months, unopened Standard, Dilution Buffer and Positive Control should be stored at -20°C or -70°C. **Detection Antibody-HRP conjugate and TMB Substrate Solution** should be stored only at 2 ~ 8˚C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Mouse serum and plasma samples may need an 1,000,000(1000K) ~ 4,000,000 (4000K)-fold dilution. A 100-fold dilution is 5 µL sample + 495 µL 1x Dilution Buffer. To make a 10,000-fold dilution is 5µL of 100-fold sample + 495 µL 1x Dilution Buffer. Finally, to make a 1,000,000-fold dilution is 5 µL of 10,000-fold sample + 495 µL 1x Dilution Buffer. Finally, to make a 2,000,000-fold dilution is 120 µL of 1,000,000-fold sample + 120 µL 1x Dilution Buffer. Finally, to make a 4,000,000-fold dilution is 80 µL of 1,000,000-fold sample + 240 µL 1x Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Dilution Buffer Concentrate (DB16F) - *If Dilution Buffer is highly viscous and crystals formed in the concentrate, warm in 27 - 30° C water bath until liquid flows more freely and the crystals have completely dissolved. DB16F cannot use directly. Must follow the dilution below:*
Dilute 60 mL of Dilution Buffer Concentrate (5-fold) into deionized or distilled water (240 mL) to prepare 300 mL of 1x Dilution Buffer.

Mouse Albumin Standard - Reconstitute the Albumin standard with 1.2 mL of 1x Dilution Buffer. This reconstitution produces a stock solution of 1000ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of 1x Dilution Buffer into tubes #1. Pipette 250 µL of 1x Dilution Buffer into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **100 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

Tube	Standard	1x Dilution Buffer	Concentration
Stock	Powder	1.2 mL	1000 ng/ml
# 1	50 µl of stock	450 µl	100 ng/ml
# 2	250 µl of 1	250 µl	50 ng/ml
# 3	250 µl of 2	250 µl	25 ng/ml
# 4	250 µl of 3	250 µl	12.5 ng/ml
# 5	250 µl of 4	250 µl	6.25 ng/ml
# 6	250 µl of 5	250 µl	3.125 ng/ml
# 7	250 µl of 6	250 µl	1.56 ng/ml

Positive Control - Reconstitute the positive control with 0.40 mL of 1x Dilution Buffer to make 10-fold concentrated positive control stock solution. Pipette 3.6 mL of 1x **Dilution Buffer DB16** into a 15 mL centrifuge tube and transfer 0.4 ml of 10-fold

concentrated stock solution to make positive control working solution.

Detection Antibody-HRP Conjugate – Pipette 11.88 mL of 1x **Dilution Buffer DB16** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *(protect from light).*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **1x Dilution Buffer** to Blank wells.
4. Add 100 µL of **Standard dilutions**, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody-HRP Conjugate working solution** to each well. Cover with plate sealer. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Substrate Solution** to each well. Incubate for 3-4 minutes. **Protect from light.** There may be fast color development, please be prepared to add stop solution immediately.
9. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well using a microplate reader set to 450 nm within 3 min.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a 4-parameter logistic (4-PL) curve fit.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

The antibodies used in this ELISA kit have been validated by immunoelectrophoresis and ELISA to react specifically with mouse serum albumin, and have essentially no reactivity with any other mouse serum proteins.

PROTEINS	CROSS-REACTIVITY (%)
Mouse Serum Albumin	100
Rat Serum Albumin	0
Human Serum Albumin	0
Mouse CRP	0
Mouse Transferrin	0
Mouse Fetuin A	0
Mouse Adiponectin	0
Mouse RBP-4	0

The serum samples from following species showed no significant cross-reactivity at 1:20000 dilution: human, bovine and pig.

TYPICAL DATA

The standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450
Blank	0 (0.049)
1.56	0.135
3.125	0.265
6.25	0.553
12.5	1.071
25	1.840
50	2.530
100	2.721

LINEARITY

To assess the linearity of the assay, pooled research mouse serum samples were diluted with 1x Dilution Buffer DB16 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (MG/ML)	RECOVERY (%)
500000X	53.276	26.638	86
1000000X	31.120	31.120	100
2000000X	15.888	31.776	102
4000000X	8.232	32.929	106

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody-HRP Conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light**.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 3-4 minutes on plate shaker at RT. **Protect from light.** Be prepared to add Stop Solution immediately.
⬇
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.

Use 5 µL of mouse serum or plasma samples to prepare 1: 1000K or 4000K dilution.

		Final Dilution
5µL of mouse sample	495 µL of 1x Dilution Buffer (DB16)	100
5µL of 100-fold diluted sample solution	495 µL of 1x Dilution Buffer (DB16)	10000
5µL of 10000-fold diluted sample solution	495 µL of 1x Dilution Buffer (DB16)	**1000000 (1000K)**
120 µL of 1000000-fold diluted sample solution	120 µL of 1x Dilution Buffer (DB16)	**2000000 (2000K)**
80 µL of 1000000-fold diluted sample solution	240 µL of 1x Dilution Buffer (DB16)	**4000000 (4000K)**

Use 10 µL of mouse serum or plasma samples to prepare 1: 1000K or 4000K dilution.

		Final Dilution
10 µL of mouse sample	995 µL of 1x Dilution Buffer (DB16)	100
10 µL of 100-fold diluted sample solution	995 µL of 1x Dilution Buffer (DB16)	10000
10 µL of 10000-fold diluted sample solution	995 µL of 1x Dilution Buffer (DB16)	**1000000 (1000K)**
120 µL of 1000000-fold (1000K) diluted sample solution	120 µL of 1x Dilution Buffer (DB16)	**2000000 (2000K)**
80 µL of 1000000-fold (1000K) diluted sample solution	240 µL of 1x Dilution Buffer (DB16)	**4000000 (4000K)**