MOUSE SERUM ALBUMIN ELISA KIT

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

PURCHASE INFORMATION:

ELISA NAME	MOUSE SERUM ALBUMIN ELISA
Catalog No.	SK00383-03
Lot No.	
Formulation	96 T
Standard Range	1-300 ng/mL
Sensitivity	1 ng/mL
Sample Volume	20 µl
Dilution Factor	800,000 (**Optimal dilutions should be determined by each laboratory for each application**)
Sample Type	Serum and EDTA Plasma
Specificity	Mouse Albumin
Intra-assay Precision	4-8%
Inter-assay Precision	8-12%
Storage	2°C – 8°C

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

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INTRODUCTION

Mouse albumin immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure Mouse Albumin in serum and plasma. It has been shown to accurately quantitate mouse albumin. Results obtained with naturally occurring albumin samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for mouse albumin.

PRINCIPLE OF THE ASSAY

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.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS 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 – 600 ng/vial of mouse albumin for calibration in a buffered protein base with preservatives; 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 mouse albumin, lyophilized	**383-03-04**	**1 vial**
Dilution Buffer Concentrate – 50mL/bottle of 10-fold concentrated buffered protein based solution with preservatives	**DB18**	**1 bottle**
Antibody-HRP Diluent Solution - 12 mL/bottle of buffered protein based solution with preservatives	**DB06**	**1 bottle**
Wash Buffer – 50 ml/bottle, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 ml/bottle of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 ml/bottle of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard and Positive Control should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Detection Antibody-HRP Conjugate 100-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
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

Serum and plasma samples may need a 800,000-fold dilution. A suggested 800,000-fold dilution is 10 µL sample + 990 µL Dilution Buffer. Following 10 µL 100x-sample + 990 µL Dilution Buffer. Following 10 µL 10,000x-sample + 790 µL Dilution Buffer. *Optimal dilutions should be determined by each laboratory for each application.*
Use polypropylene test tubes.

REAGENT PREPARATION

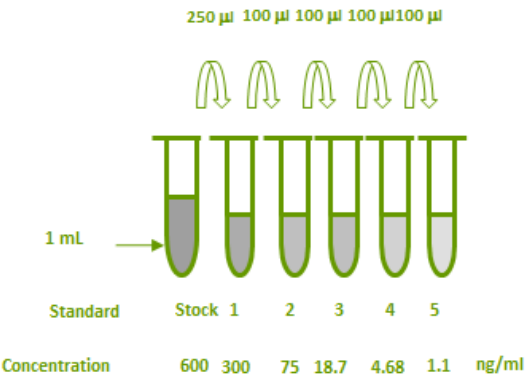
Bring all reagents to room temperature before use.

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 Wash Buffer.

Dilution 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 Dilution Buffer Concentrate into 1x PBS (450 mL) to prepare 500 mL of Dilution Buffer working solution.

Mouse Albumin Standard - Refer to vial label for reconstitution volume. Reconstitute the Albumin Standard with 1.0 ml of Dilution Buffer. This reconstitution produces a stock solution of 600 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Dilution Buffer into tube #1 and add 250 µL of stock solution to produce 300 ng/mL. Pipette 300 µL into tubes #2 to #5. Use the #1 solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 300 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 µl	600 ng/ml
# 1	250 µl of stock	250 µl	300 ng/ml
# 2	100 µl of 1	300 µl	75 ng/ml
# 3	100 µl of 2	300 µl	18.75 ng/ml
# 4	100 µl of 3	300 µl	4.688 ng/ml
# 5	100 µl of 4	300 µl	1.172 ng/ml



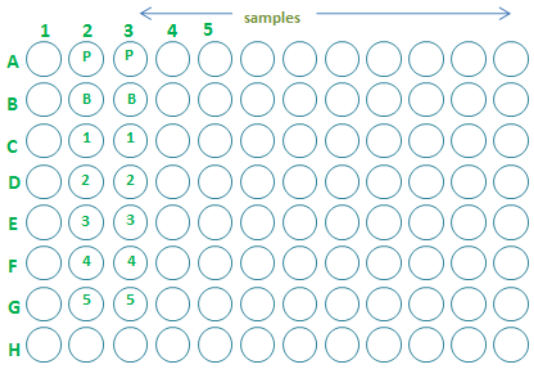
Detection Antibody HRP Conjugate – Pipette 11.88 mL of **Antibody-HRP Diluent Solution** into a 15 ml centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *Note: Detection Antibody-HRP working solution should be prepared and used immediately.*

Positive Control - Reconstitute the positive control with 2.0 mL of Dilution Buffer to make positive control working solution. *Note: Positive control should be used immediately.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well (B2, B3).
4. Add 100 µL of **Standard** (C2, C3 to G2, G3), **sample**, or **positive control** (A2, A3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **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 sealer. Incubate for 2 hours on micro-plate 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 15-60 seconds. Due to the fast color development, please pay close attention. **Protect from light.**
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 within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the albumin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified mouse albumin.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of mouse albumin was 1 ng/mL.

SPECIFICITY

This assay recognizes natural mouse serum albumin. The factors listed below were prepared at 100 µg/mL in Dilution Buffer, and assayed for cross reactivity.

Preparations of the following factors at 100 µg/mL in a mid-range mouse albumin control were assayed for interference. No significant cross-reactivity or interference was observed.

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

TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED WITH BLANK = 0.047)
1.172	0.086
4.688	0.320
18.750	1.022
75	1.762
300	1.989

*Lot No.:

** Positive Control: 35 – 75 ng/mL

LINEARITY

To assess the linearity of the assay, pooled research mouse plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (MG/ML)	RECOVERY (%)
800000X	22.882	18.3056	100
1600000X	12.046	19.2736	105
3200000X	4.820	15.424	84.3

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards

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Add 100µl of standard, samples, 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 2 hours on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to each well. Incubate **15-60 seconds** on plate shaker. **Protect from light**.

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Add 100 µl Stop Solution to each well. Read 450nm within 15 min