Mouse Albumin Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE10117

Size: 96T

Sensitivity: 0.2 pg/mL

Range: 23.4-1500 pg/mL

Usage: For the quantitative detection of mouse Albuminin concentrations in serum, plasma and urine.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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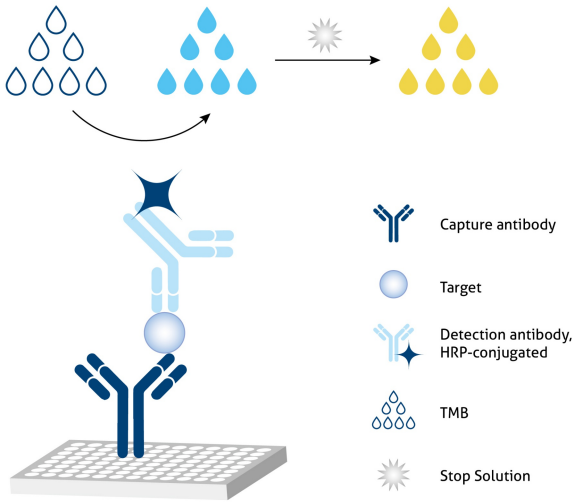
1. Background

Albumin is one of the main proteins in animal species, which plays a decisive role in the transport of various ions and in maintaining the colloidal osmotic pressure of the blood. Albumin is able to bind to almost all known drugs, as well as many nutraceuticals and toxic substances, largely determining their pharmaco-and toxicokinetics. Albumin is not only passive, but also an active participant of pharmacokinetic and toxicokinetic processes, possessing a number of enzymatic activities. Numerous experiments have shown esterase or pseudoesterase activity of albumin towards a number of endogeneous and exogeneous esters. Due to the free thiol group of Cys34, albumin can serve as a trap for reactive oxygen and nitrogen species, thus participating in redox processes. Glycated albumin makes a significant contribution to the pathogenesis of diabetes and other diseases. The interaction of albumin with blood cells, blood vessels and tissue cells outside the vascular bed is of great importance. Interactions with endothelial glycocalyx and vascular endothelial cells largely determine the integrative role of albumin.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with HRP)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 3000 pg/bottle; lyophilized	2 bottles	
Detection antibody, HRP-conjugated (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle	3 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

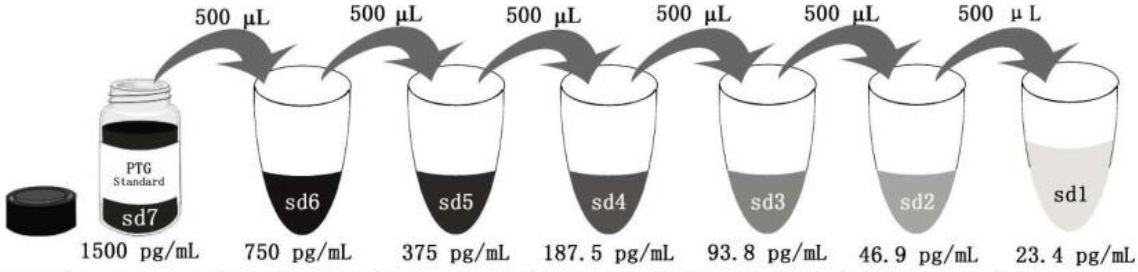
7.2 Detection Antibody, HRP-conjugated(1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution, HRP-conjugated for a few seconds prior to use).

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:8,000,000 or 1:16,000,000 is recommended for mouse serum and plasma; 1:4,000 or 1:8,000 is recommended for urine.

7.4 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.8 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.9 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.10 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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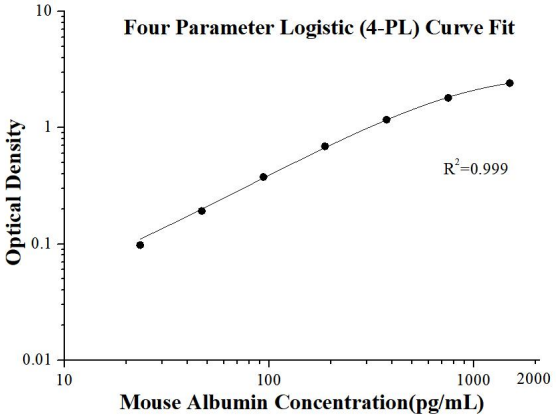
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.0432 0.046	0.0446	-
23.4	0.1398 0.1448	0.1423	0.0977
46.9	0.2255 0.247	0.23625	0.19165
93.8	0.3973 0.445	0.42115	0.37655
187.5	0.7043 0.767	0.73565	0.69105
375	1.1524 1.2776	1.215	1.1704
750	1.7801 1.9196	1.84985	1.80525
1500	2.4038 2.5298	2.4668	2.4222

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	748.6	43.1	5.8
2	20	172.6	3.4	1.9
3	20	39.1	1.5	3.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	737.3	64.6	8.8
2	24	174.4	13.3	7.6
3	24	44.4	4.2	9.4

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9.3 Recovery

The recovery of mouse Albumin spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:32,000,000	107	80-126
	1:64,000,000	106	101-111
Urine	1:8,000	94	88-101
	1:16,000	98	86-121

9.4 Sample values

Mouse serum/Urine - mouse serum and urine were evaluated for the presence of mouse Albumin in this assay.

Sample Type	Mean (**mg/mL**)	Range (**mg/mL**)
Mouse serum (n=16)	23.9	18.3-43.8

Sample Type	Mean (**µg/mL**)	Range (**µg/mL**)
Urine (n=8)	5.8	0.3-13.9

9.5 Sensitivity

The minimum detectable dose of mouse Albumin is 0.2 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The mouse serum samples were initially diluted 1:4,000,000. The urine samples were initially diluted 1:2,000.)

		Mouse serum	Urine
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	98	110
	Range (%)	97-99	100-117
1:8	Average% of Expected	104	114
	Range (%)	104-105	112-119
1:16	Average% of Expected	116	111
	Range (%)	111-122	106-116

9.7 Specificity

This assay recognizes natural and recombinant mouse Albumin.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

Albumin

Recombinant rat:

Albumin

10. References

1. Belinskaia DA, et al. Integrative Role of Albumin: Evolutionary, Biochemical and Pathophysiological Aspects. J Evol Biochem Physiol. 57(6):1419-1448 (2021).

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5. He XM and Carter DC. Atomic structure and chemistry of human serum albumin. Nature. 358(6383):209-15 (1992).

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