ab108792 Mouse Albumin ELISA Kit

For quantitative measurement of mouse albumin in plasma, serum, urine, cell culture, cell lysate and tissue samples. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab108972
(use abcam.cn/ab108972 for China, or abcam.co.jp/ab108972 for Japan)

Materials Supplied and Storage

Store kit at +4°C immediately upon receipt, apart from the Albumin Standard, SP Conjugate & Biotinylated Antibody, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Item	Quantity	Storage Condition
Albumin Microplate (12 x 8 wells)	96 wells	4°C
Albumin Standard	1 vial	4°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Mouse Albumin Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

Materials Required, Not Supplied:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilutions.

1. Reagent Preparation:

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.
- When diluting any concentrates, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

1.1 1X Diluent N

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. Store for up to 1 month at 4°C.

1.2 1X Wash Buffer

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

1.3 1X Biotinylated Albumin Detector Antibody

1.3.1 The stock Biotinylated Albumin Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated Albumin Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Albumin Antibody.

1.3.2 Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated Albumin Antibody to prepare a 1X Biotinylated Albumin Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	Total Volume 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Any remaining solution should be frozen at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated Albumin Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Albumin Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Albumin Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Albumin Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated Albumin Antibody to prepare 1X Biotinylated Albumin solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells: $\mathbf{(C_F / C_S) \times V_T = V_A}$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated Albumin Antibody: $\mathbf{V_T - V_A = V_D}$

1.3.3 First spin the Biotinylated Albumin Antibody vial to collect the contents at the bottom.

1.3.4 Add calculated amount V_A of stock Biotinylated Albumin Antibody to the calculated amount V_D of 1X Assay Diluent N. Mix gently and thoroughly.

1.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N. Any remaining solution should be frozen at -20°C.

2. Standard Preparation

- Store unused standard at -20°C after reconstitution in diluent.
- Always prepare a fresh set of standards for every use.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

2.1 Reconstitute the Albumin Stock to generate a 200 ng/mL **Standard #1**.

2.1.1 First consult the Albumin Standard vial to determine the mass of protein in the vial.

2.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the Albumin Standard vial to produce a 200 ng/mL Albumin Standard stock by using the following equation:

CS = Starting mass of Albumin Standard stock (see vial label) (ng)
CF = 200 ng/mL Albumin Standard #1 final required concentration
VD = Required volume of 1X Diluent N for reconstitution (µL)
Total required volume 1X Diluent N for resuspension: $(C_S / C_F) * 1{,}000 = V_D$

2.1.3 First briefly centrifuge the Albumin Standard Vial to collect the contents on the bottom of the tube.

2.1.4 Reconstitute the Albumin Standard vial by adding the appropriate calculated amount V_D of 1X Diluent N to the vial to generate the 200 ng/mL Albumin **Standard #1.** Mix gently and thoroughly.

2.2 Allow the reconstituted standard stock solution 200 ng/mL Albumin **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

2.3 Label five tubes #2 – 6.

2.4 Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (200 ng/ml) 4-fold with 1X Diluent N to produce 50, 12.5, 3.125, and 0.781 ng/ml solutions.

2.5 Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent N serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Mouse Albumin (ng/mL)
1	Step 2.1.4		200
2	120 µL Standard #1	360	50
3	120 µL Standard #2	360	12.5
4	120 µL Standard #3	360	3.125
5	120 µL Standard #4	360	0.781
6	N/A	360	0

3. Sample Preparation Avoid freeze-thaw cycles. Store undiluted samples at -20°C or below.

3.1 **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma. A 4,000,000-fold sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored for up to 3 months.

3.2 **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x *g* for 10 minutes and remove serum. A 4,000,000-fold sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored for up to 3 months.

3.3 **Cell Culture Supernatants:** Centrifuge cell culture media at 3,000 x g for 10 minutes to remove debris and collect supernatants. Dilute samples if necessary.

3.4 **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 2000fold sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application. The undiluted samples can be stored for up to 3 months.

3.5 **Cell Lysate:** Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 106 cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. If necessary, dilute samples into 1X Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

3.6 **Tissue:** Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into 1X Diluent N; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Guidelines for Dilutions of 100-fold or Greater *(follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

4. Assay Procedure:

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

4.1 Prepare all reagents, stock standards, and samples as directed in the previous sections. The assay is performed at room temperature (20-25°C).

4.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

4.3 Add 50 µL of Albumin Standard or sample per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

4.4 Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

4.5 Add 50 µL of Biotinylated Albumin Antibody to each well. Cover wells with a sealing tape and incubate for 1 hour.

4.6 Wash the microplate as described in step 4.4.

4.7 Add 50 µL of Streptavidin-Peroxidase Conjugate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells

with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

4.8 Wash the microplate as described in step 4.4.

4.9 Add 50 µL of Chromogen Substrate per well and incubate in ambient light for 15 minutes or until the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

4.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

4.11 Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

4.12 Analyze the data as described below.

4.12.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

4.12.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.

4.12.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Technical Support

For all technical or commercial enquiries please go to:
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