ab108791 Mouse Albumin ELISA Kit

For the quantitative measurement of mouse Albumin in plasma and serum.
For research use only - not intended for diagnostic use.
For overview, typical data and additional information please visit: www.abcam.com/ab108791
(use www.abcam.cn/ab108791 for China, or www.abcam.co.jp/ab108791 for Japan)

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the SP Conjugate, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Materials Supplied:

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 (Lyophilized)	1 vial	4°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	30 mL	4°C
Sealing Tapes	3	N/A

Materials Required, Not Supplied

These materials are not included, but will be required to successfully perform this assay:
- 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.

Reagent Preparation
- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.
- If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- Concentration of the kit components are lot-specific and the end user should always refer to the vial label.

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

1X Wash Buffer: Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly. Mix the 1x solution gently until the crystals have completely dissolved.

1X Biotinylated Albumin: Add 3 mL 1X Diluent N to the lyophilized Biotinylated Albumin vial to generate a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to dilution. From the stock solution, dilute 1:2 with Diluent N to produce a 1x working solution.
Δ Note: Any remaining solution should be frozen at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

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

1 Standard Preparation
- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1.1 Reconstitution of the Albumin Standard vial to prepare a 100 µg/mL **Stock Standard**.
1.2 First consult the Albumin Standard vial to determine the mass of protein in the vial.
1.3 Calculate the appropriate volume of 1X Diluent N to add when resuspending the Albumin Standard vial to produce a 100 µg/mL Albumin **Stock Standard** by using the following equation:
C_S = Starting mass of Albumin Standard (see vial label) (µg)
C_F = The 100 µg/mL Albumin **Stock Standard** final required concentration
V_D = Required volume of 1X Diluent N for reconstitution (µL)
Calculate total required volume 1X Diluent N for resuspension:
$(C_S / C_F) \times 1{,}000 = V_D$

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Mouse Albumin (µg/mL)
1	Step 1.1		100
2	120 µL Standard #1	120	50
3	120 µL Standard #2	120	25
4	120 µL Standard #3	120	12.5
5	120 µL Standard #4	120	6.25
6	120 µL Standard #5	120	3.125
7	120 µL Standard #6	120	1.563
8 (Blank)	N/A	120	0

2 Sample Preparation

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x g for 10 minutes. Dilute samples 1:7500 into Diluent N and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant).

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. Dilute samples 1:7500 into Diluent N and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please 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*

3 Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

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, working 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 and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

4.3 Add 25 µL of Albumin Standard or sample per well and immediately add 25 µL of 1X Biotinylated Albumin to each well (on top of the standard or sample) and mix gently. Cover wells with a sealing tape and incubate for one hour at room temperature. Start the timer after the last sample addition.

4.4 Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

4.5 Add 50 µL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

4.6 Wash microplate as described above.

4.7 Add 50 µL of Chromogen Substrate per well and incubate for 20 minutes in ambient light or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

4.8 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

4.9 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.

4.10 Analyze the data as described below.

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- 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.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

5 Typical Sample Values

SENSITIVITY –
The minimum detectable does (MDD) of Albumin is typical ~ 1.4 µg/ml.

PRECISION -

	Intra-assay Precision	Inter-assay Precision
CV (%)	5.2	9.6

RECOVERY –
Standard Added Value: 1.563 – 25 µg/ml
Recovery (%): 88 - 113 %
Average Recovery (%): 96%

6 Species Reactivity

Species	% Cross Reactivity
Bovine, Human, Swine, Canine, Monkey, Rabbit, Equine	None
Rat	<10
Mouse	100

7 Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4oC
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

Technical Support
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