ab108788 – Albumin Human ELISA Kit

For the quantitative measurement of Human Albumin in urine, saliva, milk, CSF, cell culture supernatants, cell lysate and tissue samples.
This product is for research use only and is not intended for diagnostic use.

Storage and Stability:

Store kit at +4°C immediately upon receipt, apart from the 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.

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
25X Biotinylated Human 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

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel)
- Deionized or distilled reagent grade water

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 the 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.

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.

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

25X Biotinylated Albumin Detector Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 1:25 with Diluent N to produce a 1x solution. The undiluted antibody should be stored at -20°C.

100X 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. The undiluted conjugate should be stored at -20°C.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the Albumin Stock to generate a 200 ng/mL **Standard #1**.
2. Any remaining Albumin Standard stock solution should be frozen at -20°C.
3. First consult the Albumin Standard vial to determine the mass of protein in the vial.
4. 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 (see vial label) (ng)
   CF = The 200 ng/mL Albumin Standard #1 final required concentration
   VD = Required volume of 1X Diluent N for reconstitution (µL)
   Calculate total required volume 1X Diluent N for resuspension:
   (CS / CF) x 1,000 = VD

Example: **Δ Note: This example is for demonstration purposes only.** Please remember to check your standard vial for the actual amount of standard provided.
$\mathbf{C_S}$ = 400 ng of Albumin Standard in vial
$\mathbf{C_F}$ = 200 ng/mL Albumin Standard #1 final concentration
$\mathbf{V_D}$ = Required volume of 1X Diluent N for reconstitution (400 ng / 200 ng/mL) x 1,000 = 2,000 µL

5. Reconstitute the Albumin Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 200 ng/mL Albumin Standard #1. Mix gently and thoroughly.
6. Allow the reconstituted 200 ng/mL Albumin Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
7. Label five tubes #2 – 8.
8. Add 120 µL of 1X Diluent N to tube #2 – 8.
9. To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently.
10. To prepare Standard #3, add 120 µL of the Standard #2 into tube #3 and mix gently.
11. 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)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 5				200.0
2	120	120	240	200.0	100.0
3	120	120	240	100.0	50.00
4	120	120	240	50.00	25.00
5	120	120	240	25.00	12.50
6	120	120	240	12.50	6.250
7	120	120	240	6.250	3.125
8	-	120	120	-	0

Sample Preparation

Cell Culture Supernatants: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into 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.

Urine: Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. A 1:200 sample dilution is suggested into Diluent N or within the range of 1:50 – 1:400; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute saliva samples into 1X Diluent N and assay, 1:200 is recommended. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. A 1:6000 sample dilution is suggested into Diluent N or within the range of 1:1000 – 1:12000; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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 10^6 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. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Cerebrospinal fluid: Collect cerebrospinal fluid (CSF) using sample tube. Centrifuge samples at 3,000 x *g* for 10 minutes. Dilute CSF samples 1:2000 - 1:10 000 into 1X Diluent N and assay. The undiluted samples can be stored at -80°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

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.

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).

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.

1. Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).
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.
3. Add 50 µL of Albumin Standard or sample per well. Cover wells with a sealing tape and incubate for 1 hour. Start the timer after the last addition.
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.
5. Add 50 µL of 1X Biotinylated Albumin Antibody to each well and incubate for 30 minutes.
6. Wash microplate as described above.
7. 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.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate per well and incubate in ambient light for about 25 minutes or until the optimal blue colour density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
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.

Calculation

- 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.

Typical Sample Values

Sensitivity:

The minimum detectable dose (MDD) of Albumin is typically 0.79 ng/ml.

Precision:

	Intra-assay Precision	Inter-Assay Precision
CV (%)	5.9	10.4

Recovery:

Standard Added Value	12.5 – 100 ng/ml
Recovery (%)	89 – 110%
Average Recovery (%)	99%

Linearity of Dilution

- Linearity of dilution is determined based on interpolated values from the standard curve.
- Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Average Percentage of Expected Value (%)	
Dilution Factor	Milk
1:3,000	107
1:6,000	102
1:12,000	91

Troubleshooting

	Reason	Solution
Poor Standard Curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly re-suspend 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)
High Background/Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)