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ab108789
Rat Albumin ELISA Kit

For the quantitative measurement of rat albumin in plasma, serum, urine samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Rat Albumin *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Albumin in plasma, serum and urine samples.

An Albumin specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently an Albumin specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Albumin captured in plate.

Albumin, a serum hepatic protein, is the most abundant protein in serum. It contributes to the maintenance of oncotic pressure as well as the transport of hydrophobic molecules. Serum Albumin level has been linked in clinical practice to several diseases. Low Albumin levels can suggest liver disease, kidney disease, inflammation, shock, and malnutrition. On the other hand, high Albumin levels usually reflect dehydration.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to appropriate wells.

Incubate at room temperature.

Wash and add prepared biotin antibody to each well. Incubate at room temperature.

Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.

Add Chromogen Substrate to each well. Incubate at room temperature

Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. 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.

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.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. 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 Rat 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

7. 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.
- 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.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. 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.
- If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

9.1 1X Diluent N

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly.

Δ Note: Store for up to 30 days month at 4°C. When diluting the concentrate, 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.

9.2 1X Wash Buffer

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

Δ Note: When diluting the concentrate, 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.

9.3 1X Biotinylated Albumin Detector Antibody

9.3.1 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.

9.3.2 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	(V_T) Total Volume of 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640

8	64	3,520
10	80	4,400
12	96	5,280

Δ Note: 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:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated Albumin Antibody:

$$V_T - V_A = V_D$$

Example:

Δ Note: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 50X Biotinylated Albumin Antibody stock

C_F = 1X Biotinylated Albumin Antibody solution for use in the assay procedure

V_T = 3,520 µL (8 well strips or 64 wells)

$$(1X/50X) \times 3{,}520\ \mu L = 70.4\ \mu L$$

$$3{,}520\ \mu L - 70.4\ \mu L = 3{,}449.6\ \mu L$$

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

9.3.4 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.

9.4 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.

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

10. 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).

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

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

10.1.2 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:

C_S = Starting mass of Albumin Standard (see vial label) (ng)

C_F = The 200 ng/mL Albumin **Standard #1** final required concentration

V_D = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 220 ng of Albumin Standard in vial

C_F = 200 ng/mL Albumin **Standard #1** final concentration

V_D = Required volume of 1X Diluent N for reconstitution

(220 ng / 200 ng/mL) x 1,000 = 1,100 µL

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

10.1.4 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.

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

10.3 Label five tubes #2 – 6.

10.4 Add 360 µL of 1X Diluent N to tube #2 – 6.

10.5 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.6 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.7 Using the table below as a guide, prepare subsequent serial dilutions.

10.8 1X Diluent N serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Rat Albumin (ng/mL)
1	Step 10.1		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 (Blank)	N/A	360	0

11. Sample Preparation

11.1 Urine:

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute samples within a range of 1:100 - 1:4000 into 1X Diluent N, with a recommended starting dilution of 1:800, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 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 4000000-fold sample dilution is suggested into 1X Diluent N; 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.

11.3 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 4000000-fold sample dilution is suggested into 1X Diluent N; 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.
depending on application needs. The undiluted samples can be stored at -80°C. 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*

12. 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.

13. 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.

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

13.2 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.

13.3 Add 50 µL of Albumin Standard to each 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 one hour. Start the timer after the last addition.

13.4 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.

13.5 Add 50 µL of 1X Biotinylated Albumin Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles may formed. Cover wells with a sealing tape and incubate for 30 minutes.

13.6 Wash microplate as described above.

13.7 Add 50 µL of 1X SP Conjugate to each 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.

13.8 Wash microplate as described above.

13.9 Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for about 30 minutes or till the optimal blue color density develops.

13.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.

13.11 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.

13.12 Analyze the data as described below.

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

13.12.2 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.

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

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

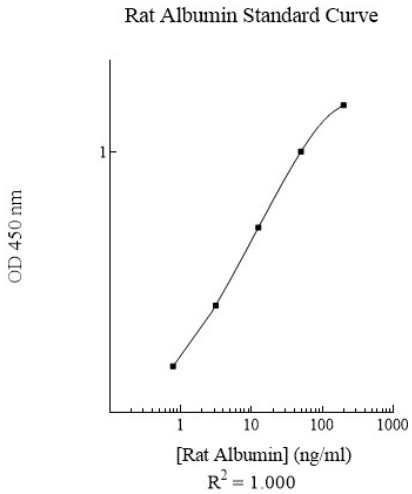

Figure 1. Example of Albumin standard curve. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of Albumin is typically about 0.39 ng/ml.

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	5.4	10.6

RECOVERY –

Standard Added Value	3.125 – 50 ng/ml
Recovery (%)	86-113 %
Average Recovery (%)	97 %

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.

Plasma samples were serially-diluted to test for linearity.

Dilution Factor	Plasma	Serum
2000000x	93%	92%
4000000x	98%	99%
8000000x	106%	109%

16. Assay Specificity

This kit recognizes Albumin in urine and cell culture supernatant samples.

17. Species Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Equine	None
Monkey	None
Mouse	5
Swine	None
Rabbit	None
Human	None

18. 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 4°C
	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)

Problem	Cause	Solution
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

19. Notes

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)