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ab108895 – PreAlbumin (Transthyretin) Human ELISA Kit

For the quantitative measurement of Human PreAlbumin (Transthyretin) in plasma, serum, milk, urine, saliva, CSF samples.

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

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1. Overview

Abcam's PreAlbumin (Transthyretin) Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of PreAlbumin concentrations in plasma, serum, milk, urine, saliva, CSF, cell culture, cell lysate, and tissue samples.

A PreAlbumin specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a PreAlbumin 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 PreAlbumin captured in plate.

PreAlbumin (Transthyretin) is a hepatic secretory protein thought to be important in the evaluation of nutritional deficiency and nutrition support. PreAlbumin plays important physiological roles as a transporter of thyroxine and retinol-binding protein. Decreased PreAlbumin levels have been suggested to associate with malnutrition and chronic kidney disease.

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.
- Modifications to the kit components or procedures may result in loss of performance.

4. Storage and Stability

Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.

Store Standard, SP Conjugate, and Biotinylated Antibody at -20°C.

Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.

Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

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	Amount	Storage Condition (Before Preparation)
PreAlbumin Microplate (12 X 8 well strips)	96 wells	4°C
PreAlbumin Standard	1 vial	-20°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Human PreAlbumin 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:

- 1 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.
- 8 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.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.

9. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. 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. Store for up to 1 month at 4°C.

9.2 1X Wash Buffer

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

9.3 100X Streptavidin-Peroxidase Conjugate:

Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent N to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

9.4 1X Biotinylated PreAlbumin Detector Antibody

- The stock Biotinylated PreAlbumin Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated PreAlbumin Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated PreAlbumin Antibody.
- Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated PreAlbumin Antibody to prepare a 1X Biotinylated PreAlbumin 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

10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Any remaining standard should be stored at -20°C after reconstitution and used within 10 days.
- This procedure

10.1 Reconstitution of the PreAlbumin Standard vial to prepare a 62.5 ng/mL PreAlbumin Stock Standard:

- First consult the PreAlbumin Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of 1X Diluent N to add when resuspending the PreAlbumin Standard vial to produce a 62.5 ng/mL PreAlbumin Stock Standard by using the following equation:

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

C_F = 62.5 ng/mL PreAlbumin 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:

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

Example:

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

CS = 50 ng of PreAlbumin Standard in vial

CF = 62.5 ng/mL PreAlbumin Stock Standard final concentration

VD = Required volume of 1X Diluent N for reconstitution

(50 ng / 62.5 ng/mL) x 1,000 = 800 µL

10.2 First briefly spin the PreAlbumin Standard Vial to collect the contents on the bottom of the tube.

10.3 Reconstitute the PreAlbumin Standard vial by adding the appropriate calculated amount V_D of 1X Diluent N to the vial to generate the 62.5 ng/mL PreAlbumin Stock Standard. Mix gently and thoroughly.

10.4 Allow the reconstituted 62.5 ng/mL PreAlbumin Stock Standard to sit for 10 minutes with gentle agitation prior to making subsequent dilutions.

10.5 Label seven tubes #1-6

10.6 Add 240 µL 1X Diluent N to tube #1. Add 240 µL of the PreAlbumin Stock Standard (62.5 ng/ml) and mix gently.

10.7 Add 360 µL of 1X Diluent N to tubes #2 – 6.

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

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

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

10.11 1X Diluent N serves as the zero standard, 0 ng/mL (tube #6).

Standard #	Volume to Dilute (µL)	Volume Diluent N (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	240 µl of Stock Standard (62.5 ng/ml)	240	480	62.5	31.25
2	120	360	480	31.25	7.813
3	120	360	480	7.813	1.953
4	120	360	480	1.953	0.488
5	120	360	480	0.488	0.122
6	-	360	360	-	0

11. Sample Preparation

11.1 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 1:80,000 into 1X 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).

11.2 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. Dilute samples 1:80,000 into 1X Diluent N and assay. The undiluted samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 Milk

Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Milk dilution is suggested at 1:1000 in 1X Diluent N or within the range of 100X – 1000X; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.4 Urine

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. The sample is suggested for use at 1X or within the range of 100X – 10,000X; 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.

11.5 Saliva

Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:100 into 1X Diluent N or within the range of 10X – 1000X; however, the 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.

11.6 CSF

Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:4000 into 1X Diluent N or within the range of 400X – 40,000X; however, the user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

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*

Refer to Dilution Guidelines for further instruction.

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 plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, standard solutions and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18 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 PreAlbumin Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample 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 PreAlbumin Antibody to each well. Cover wells with a sealing tape and incubate for 1 hour

13.6 Wash microplate as described above.

13.7 Add 50 µL of 1X SP Conjugate to each well. 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 per well and incubate in ambient light for 15 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.

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

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.

14. Calculations

Calculate the mean value of the 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 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.

15. Typical Data

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

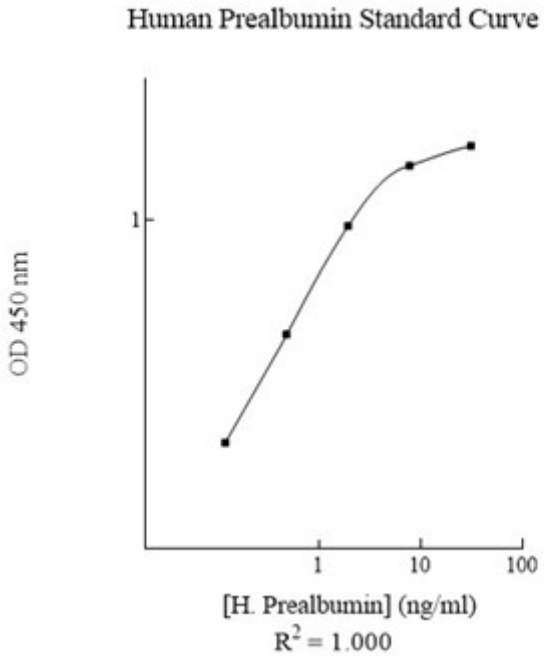

Figure 1. Example of PreAlbumin standard curve.

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of PreAlbumin is typically 49 pg/ml.

RECOVERY –

Standard Added Value: 0.5 – 7.5 ng/mL

Recovery %: 87 – 112.

Average Recovery %: 98

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 Dilution	Average % Expected Value
1.40,000	102
1:80,000	98
1:160,000	100

Serum Dilution	Average % Expected Value
1:40,000	110
1:80,000	105
1:160,000	90

PRECISION –

	Intra-assay Precision	Inter-Assay Precision
CV (%)	5.7	9.4

17. Assay Specificity

Species	Cross-Reactivity (%)
Canine	None
Bovine	None
Equine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None
Protein	**Cross-Reactivity (%)**
Human Albumin	None

18. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.

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)