

Human Prealbumin ELISA Kit

Introduction

Prealbumin (transthyretin) is a hepatic secretory protein thought to be important in the evaluation of nutritional deficiency and nutrition support (1). Prealbumin plays important physiological roles as a transporter of thyroxine and retinol-binding protein (2). Decreased prealbumin levels have been suggested to associate with malnutrition (3), and chronic kidney disease (4).

Principal of the Assay

The Human Prealbumin ELISA kit is designed for detection of human Prealbumin in plasma, serum, urine, saliva, milk, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures prealbumin in less than 4 hours. A polyclonal antibody specific for prealbumin has been pre-coated onto a microplate. Prealbumin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for prealbumin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution

Reagents

- **Prealbumin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human prealbumin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Prealbumin Standard:** Human Prealbumin in a buffered protein base (250 ng lyophilized).

- **Biotinylated Prealbumin Antibody (70x):** A 70-fold concentrated biotinylated polyclonal antibody against prealbumin (105 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- 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.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:40000 into MIX Diluent. If necessary, dilute samples further to within the range of 1:50000 to 1:100000 into MIX Diluent. 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 anticoagulant.)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:40000 into MIX Diluent. If necessary, dilute samples further to within the range of 1:50000 to 1:100000 into MIX Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Dilute samples 1:10 into MIX Diluent. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:100 into MIX Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Milk:** Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes. Milk dilution is suggested at 1:1000 in MIX Diluent; however, the user should determine the optimal dilution factor. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Prealbumin Standard:** Reconstitute the 250 ng of human Prealbumin Standard with 4 ml of MIX Diluent to generate a stock solution of 62.5 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by diluting the Standard Stock (62.5 ng/ml) 1:2 with MIX Diluent to produce the Standard solution of 31.25 ng/ml. Prepare duplicate or triplicate standard points by diluting the solution (31.25 ng/ml) 1:4 with MIX Diluent to produce solution of 7.82, 1.95, 0.49, 0.12 and 0.03 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[Prealbumin] (ng/ml)
P1	1 part Stock (62.5 ng/ml) + 1 part MIX Diluent	31.25
P2	1 part P1 + 3 parts MIX Diluent	7.82
P3	1 part P2 + 3 parts MIX Diluent	1.95
P4	1 part P3 + 3 parts MIX Diluent	0.49
P5	1 part P4 + 3 parts MIX Diluent	0.12
P6	1 part P5 + 3 parts MIX Diluent	0.03
P7	MIX Diluent	0.00

- **Biotinylated Prealbumin Antibody (70x):** Spin down the SP Conjugate briefly and dilute the desired amount of the antibody 1:70 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- 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.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.

If using a machine wash six times with 300 μ l of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.

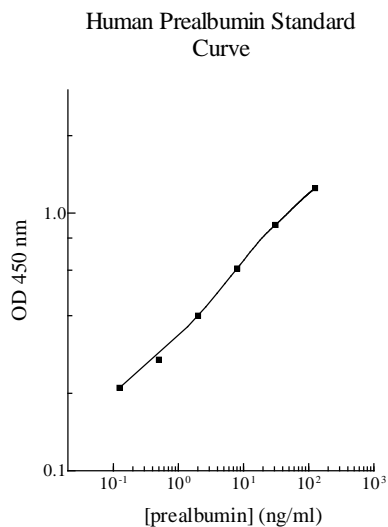
- Add 50 μ l of Biotinylated Prealbumin Antibody to each well and incubate for one hour.
- Wash the microplate as described above.
- Add 50 μ l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μ l of Chromogen Substrate per well and incubate for about 20 minutes 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.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.
- 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.

Data Analysis

- 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 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.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of prealbumin is typically 0.02 ng/ml.

- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.4% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
20000x	89%	91%
40000x	96%	97%
80000x	102%	99%

	Average Percentage of Expected Value	
Sample Dilution	Saliva	
50x	87%	
100x	95%	
200x	104%	

	Average Percentage of Expected Value	
Sample Dilution	Milk	
500x	90%	
1000x	96%	
2000x	102%	

Recovery

Standard Added Value	0.2 – 20 ng/ml
Recovery %	84 – 112%
Average Recovery %	97%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Monkey	0.1%
Mouse	0.1%
Rat	None
Swine	None
Rabbit	None
Bovine	None

References

- (1) Chertow GM *et al.* (2005) *Kidney Int.* 68(6): 2794-800
- (2) Hamilton JA. *et al.*(2001) *Cell Mol Life Sci.* 58(10):1491-521
- (3) Beck FK. *et al.* (2002) *Am Fam Physician.* 15; 65(8): 1575-8
- (4) Kaysen GA. *et al.* (2004) *J Am Soc Nephrol* 15 (3): 538-48

Version 5.8