NOTE: Revisions to Preparation of Standards and Samples

Product Manual

Human Albumin ELISA Kit

Catalog Number

STA-383 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Human serum albumin (HSA) is the most abundant protein in human blood plasma. Albumin constitutes about half of the blood serum protein. It is soluble and monomeric. The typical albumin concentration in blood is around 5 g/dL (50 mg/mL). It is produced in the liver as preproalbumin, which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin. Albumin transports hormones, fatty acids, and other compounds, buffers pH, and maintains osmotic pressure.

Cell Biolabs' Human Albumin ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human albumin in plasma, serum, urine or other biological fluid samples. The kit has a detection sensitivity limit of 5 ng/mL human albumin. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-362: Human ApoAI ELISA Kit
2. STA-363: Human ApoAII ELISA Kit
3. STA-364: Human ApoCI ELISA Kit
4. STA-365: Human ApoCII ELISA Kit
5. STA-366: Human ApoCIII ELISA Kit
6. STA-367: Human ApoE ELISA Kit
7. STA-368: Human ApoB ELISA Kit
8. STA-369: Human Oxidized LDL ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-HSA Antibody Coated Plate (Part No. 238301): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-HSA Antibody (1000X) (Part No. 238302): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. HSA Standard (Part No. 238303): One 50 µL vial of 40 µg/mL Human Serum Albumin in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum, Urine or Other Biological Fluids
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Biotinylated Anti-HSA Antibody and the HSA Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-HSA Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-HSA antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:2000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human Albumin Standard

Prepare a dilution series of human serum albumin standards in the concentration range of 0 to 400 ng/mL in Assay Diluent (Table 1).

Standard Tubes	40 µg/mL Human Serum Albumin Standard (µL)	Assay Diluent (µL)	Human Serum Albumin (ng/mL)
1	10	990	400
2	500 of Tube #1	500	200
3	500 of Tube #2	500	100
4	500 of Tube #3	500	50
5	500 of Tube #4	500	25
6	500 of Tube #5	500	12.5
7	500 of Tube #6	500	6.25
8	0	500	0

Table 1. Preparation of Human Serum Albumin Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge at 4ºC for 10 minutes at 1000 g. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require about 1,000,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. Prepare a serial dilution of each sample according to Table 2 below, mixing each dilution well before continuing to the next dilution step.
- Serum: Harvest serum and centrifuge at 4ºC for 10 minutes at 1000 g. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require about 1,000,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. Prepare a serial dilution of each sample according to Table 2 below, mixing each dilution well before continuing to the next dilution step.

Sample Tubes	Plasma or Serum Sample	PBS with 0.1% BSA	Effective Dilution
1	5 µL	5 mL	1:1000
2	5 µL of Tube #1	5 mL	1:1,000,000

Table 2. Preparation of 1:1,000,000 dilution of plasma or serum samples.

- Urine: Harvest urine and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Normal urine samples require about 1000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months.

Assay Protocol

1. Prepare dilutions of plasma, serum, urine or other biological fluid samples in PBS containing 0.1% BSA.
2. Add 100 µL of human albumin unknown sample or standard to the Anti-HSA Antibody Coated Plate. Each human albumin unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-HSA antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.

6. Wash the strip wells 3 times according to step 4 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
9. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human Albumin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

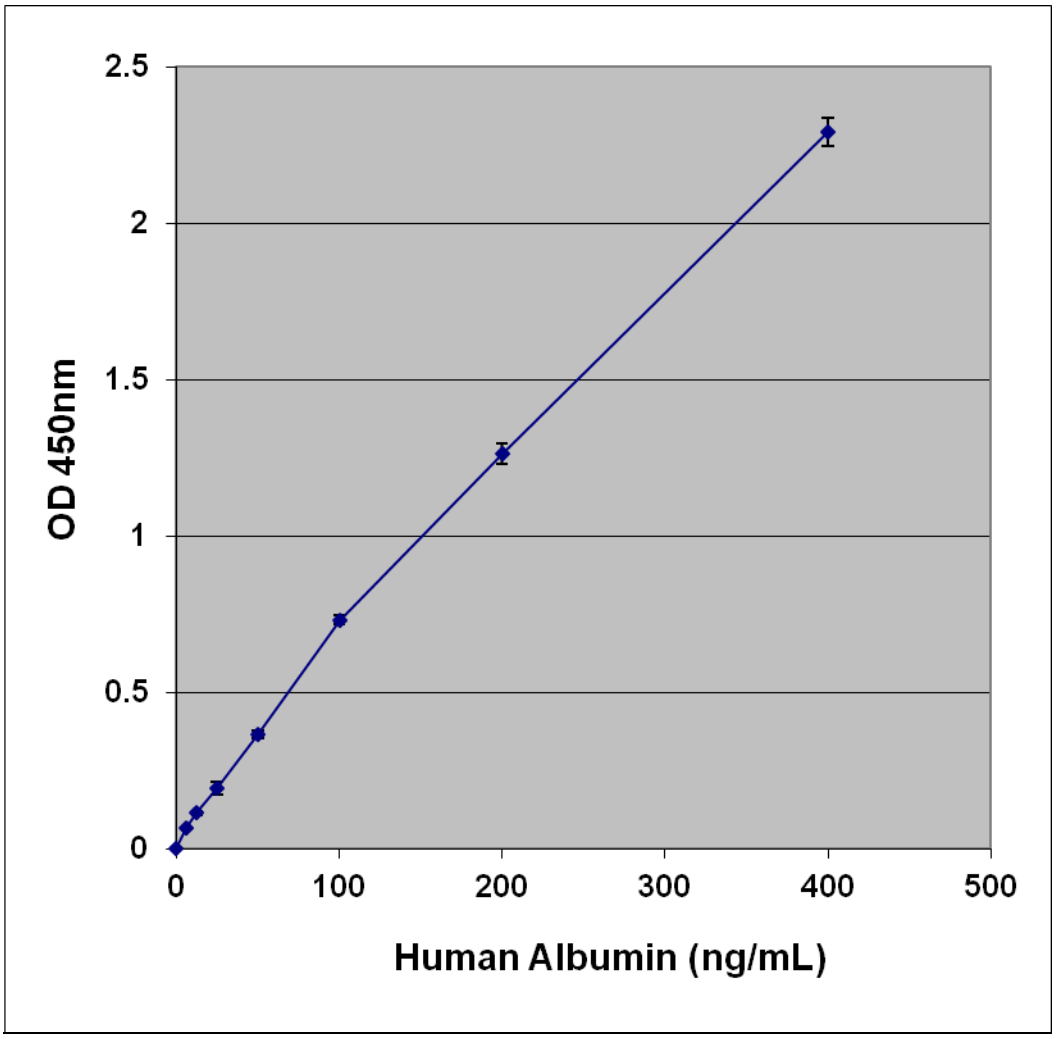

Figure 1: Human Albumin ELISA Standard Curve.

References

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Recent Product Citations

1. Hyams, J.S. et al. (2017). Factors associated with early outcomes following standardised therapy in children with ulcerative colitis (PROTECT): a multicentre inception cohort study. *Lancet Gastroenterol Hepatol*. **2**(12):855-868. doi: 10.1016/S2468-1253(17)30252-2.
2. Gabr, S.A. et al (2017). Biological activities of ginger against cadmium-induced renal toxicity. *Saudi Journal of Biological Sciences*. In Press.
3. Yang, J. et al. (2017). Generation of Human Liver Chimeric Mice with Hepatocytes from Familial Hypercholesterolemia Induced Pluripotent Stem Cells. *Stem Cell Reports*. **8**(3):605-618. doi: 10.1016/j.stemcr.2017.01.027.
4. Bielewicz, J. et al. (2016). Worse neurological state during acute ischemic stroke is associated with a decrease in serum albumin levels. *J Mol Neurosci.* doi:10.1007/s12031-015-0705-4.

Warranty

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