Product Manual

Human Alpha 1 Acid Glycoprotein ELISA Kit

Catalog Number

PRB-5044 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Alpha 1 Acid Glycoprotein (α1AG), also known as Orosomucoid, is an acute phase protein produced in liver cells and found at high levels in human plasma. α1AG levels can change in response to certain drugs, some disease states such as HIV infection, and pregnancy. α1AG carries basic molecules (as opposed to acidic or neutral), protease inhibitors, or steroids in the blood. Low α1AG levels can bind and activate the thyrotropin receptor causing an increase in cyclic AMP levels. In contrast, higher levels of α1AG inhibit signaling of the thyrotropin receptor. In mice, knockout of the α1AG gene causes abnormal energy homeostasis including increases in fat mass and body weight. Furthermore, obese mice have significantly raised levels of the protein in adipose tissue, blood, and liver. α1AG has been recognized as one of four possible circulating biomarkers for estimating the five-year risk of all-cause mortality.

Cell Biolabs' Human α1AG ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human α1AG in plasma, serum, urine, cell or tissue lysate samples. The kit has a detection sensitivity limit of 30 pg/mL human α1AG. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5039: Human Haptoglobin ELISA Kit
3. PRB-5041: Human Ceruloplasmin ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human α1AG Antibody Coated Plate (Part No. 50441B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-α1AG Antibody (200X) (Part No. 50442C): One 50 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. Human α1AG Standard (Part No. 50443D): One 50 µL vial of 200 ng/mL Human α1AG.

Materials Not Supplied

1. Plasma, serum, cell or tissue lysate
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 Human α1AG Standard at -80ºC and store the Biotinylated Anti-α1AG Antibody (200X) 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 to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-α1AG Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute Biotinylated Anti-α1AG Antibody 1:200 or dilute the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human α1AG Standard

Prepare a dilution series of human α1AG standards in the concentration range of 0 to 2000 pg/mL into Assay Diluent (Table 1).

Standard Tubes	200 ng/mL Human α1AG Standard (µL)	Assay Diluent (µL)	Human α1AG (pg/mL)
1	8	792	2000
2	400 of Tube #1	400	1000
3	400 of Tube #2	400	500
4	400 of Tube #3	400	250
5	400 of Tube #4	400	125
6	400 of Tube #5	400	62.5
7	400 of Tube #6	400	31.25
8	0	400	0

Table 1. Preparation of Human α1AG 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 an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require about 100,000 to 1,000,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. For example, for a 200,000 fold dilution prepare a serial dilution of each

sample according to Table 2 below, mixing each dilution well before continuing to the next dilution step.

- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require about 100,000 to 1,000,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. For example, for a 200,000 fold dilution 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	1000 µL	1:200
2	5 µL of Tube #1	4995 µL	1:200,000

Table 2. Preparation of 1:200,000 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. Dilute samples in PBS containing 0.1% BSA as needed.
- 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. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of human α1AG unknown sample or standard to the Anti-Human α1AG Antibody Coated Plate. Each human α1AG unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. 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.
4. Add 100 µL of the diluted Biotinylated Anti-α1AG Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.

6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. 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.
9. 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).
10. 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 Alpha 1 Acid Glycoprotein ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

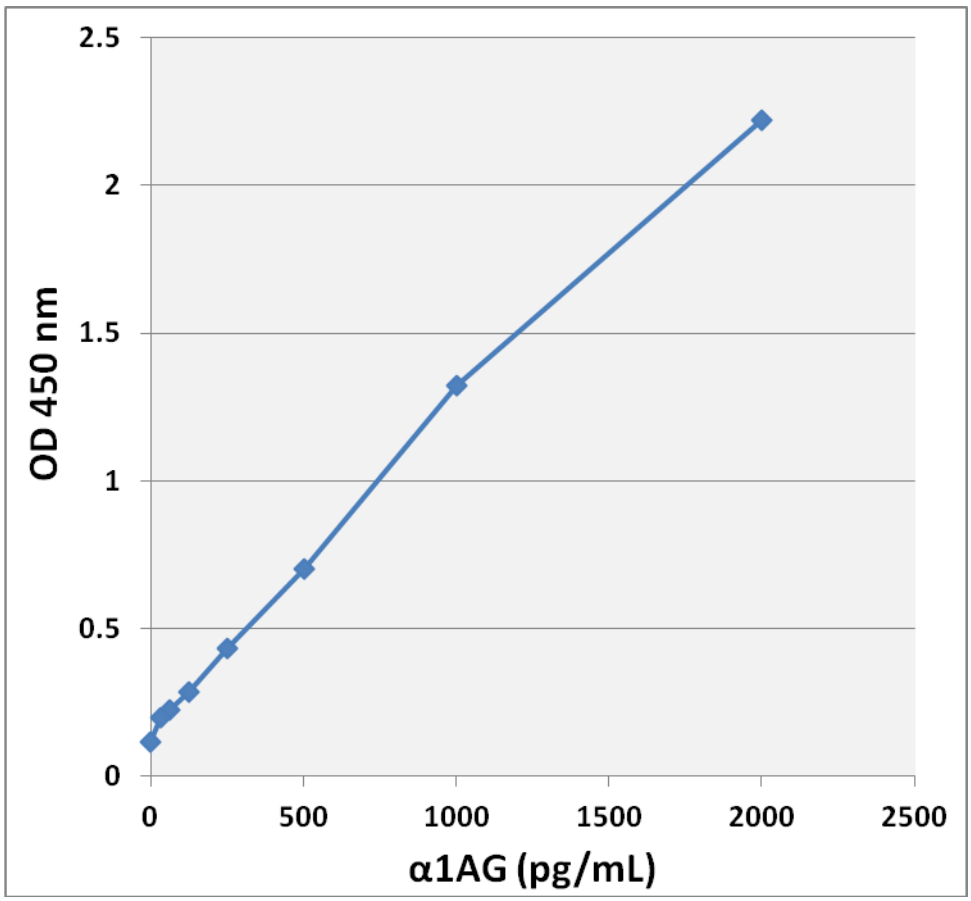

Figure 1: Human α1AG ELISA Standard Curve.

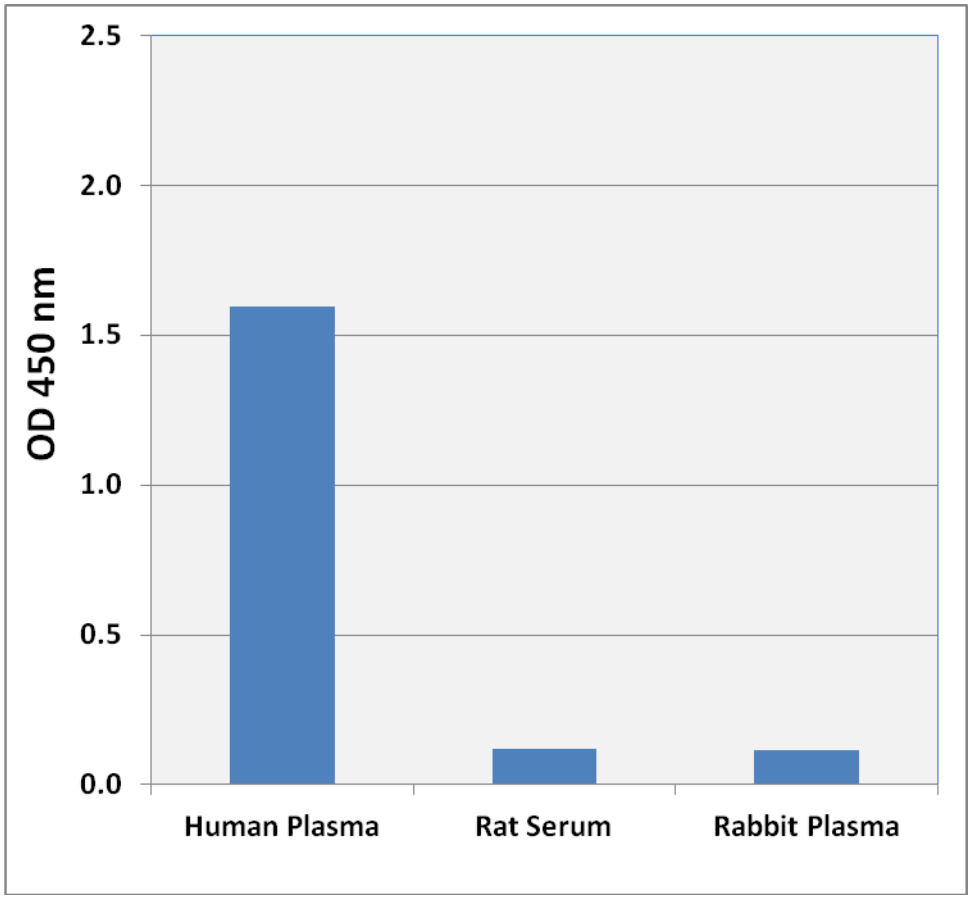

Figure 2: Detection of α1AG in Plasma. Each plasma or serum sample was diluted 1:125,000 fold according to the protocol above and then tested using the Human Alpha 1 Acid Glycoprotein ELISA Kit.

References

1. Colombo S, Buclin T, Décosterd LA, Telenti A, Furrer H, Lee BL, Biollaz J, Eap CB (2006). *Clin. Pharmacol. Ther.* **80**: 307–318
2. Urien S, Brée F, Testa B, Tillement JP (1991). *Biochem. J.* **280**: 277–280.
3. Zimmermann-Belsing, T; Rasmussen, A. K.; Feldt-Rasmussen, U; Bøg-Hansen, T. C. (2002). *Mol. Cel. Endocrin.* **188**: 241–251
4. Dietrich, J. W.; Landgrafe, G; Fotiadou, E. H. (2012). *J. Thyroid Res.* **2012**: 351864
5. Fischer, Kettunen, Würtz; et al. (2014). *PLoS Medicine*. **11**: e1001606
6. Luo Z, Lei H, Sun Y, Liu X, and Su DF. (2015) *J. Physiol. Biochem*. **71**:329-340.
7. Sun Y, Yang Y, Qin Z, Cai J, Guo X, Tang Y, Wan J, Su DF, Liu X. (2016). *Diabetes* **65**:1630-1641

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's

exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com