Product Manual

Human Alpha 2 Macroglobulin ELISA Kit

Catalog Number

PRB-5033 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Alpha 2-Macroglobulin (α2M) is a 718-kDa glycoprotein composed of four identical subunits. It is located in extracellular spaces as well as plasma. While α2M was first demonstrated to be a broad spectrum inhibitor of proteases, α2M also contains a binding site for low density lipoprotein receptor-related protein (LRP-1). Because LRP-1 functions as an endocytic receptor, α2M bound to protease is rapidly removed from the blood. In addition to its role as an inhibitor and clearance factor for proteases, α2M binds and shuttles specific growth factors, including transforming growth factor (TGF), platelet-derived growth factor-BB (PDGF-BB), nerve growth factor (NGF), and neurotrophin-4. α2M may additionally inhibit the activity of growth factors or enhance growth factor delivery to cell signaling receptors.

Cell Biolabs' Human Alpha 2-Macroglobulin ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human α2M in plasma, serum, cell or tissue lysate samples. The kit has a detection sensitivity limit of 1 ng/mL human α2M. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-368: Human ApoB ELISA Kit
3. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-385: PCSK9 ELISA Kit
5. STA-386: Human LDLR ELISA Kit
6. STA-387: Human LOX-1 ELISA Kit
7. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
8. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human α2M Antibody Coated Plate (Part No. 50331B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Human α2M Antibody (200X) (Part No. 50332C): 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 α2M Standard (Part No. 50333D): One 50 µL vial of 4 µg/mL Human α2M.

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 α2M Standard at -80ºC and the Biotinylated Anti-Human α2M Antibody 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-Human α2M Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human α2M antibody 1:200 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human α2M Standard

Prepare a dilution series of human α2M standards in the concentration range of 0 to 40,000 pg/mL into Assay Diluent (Table 1).

Standard Tubes	4 µg/mL Human α2M Standard (µL)	Assay Diluent (µL)	Human α2M (ng/mL)
1	8	792	40
2	400 of Tube #1	400	20
3	400 of Tube #2	400	10
4	400 of Tube #3	400	5
5	400 of Tube #4	400	2.5
6	400 of Tube #5	400	1.25
7	400 of Tube #6	400	0.625
8	0	400	0

Table 1. Preparation of Human α2M 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 5,000-20,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. For example, for a 10,000 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 5,000-20,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. For example, for a 10,000 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	500 µL	1:100
2	5 µL of Tube #1	500 µL	1:10,000

Table 2. Preparation of 1:10,000 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 α2M unknown sample or standard to the Anti-Human α2M Antibody Coated Plate. Each human α2M 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-Human α2M 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 α2M ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

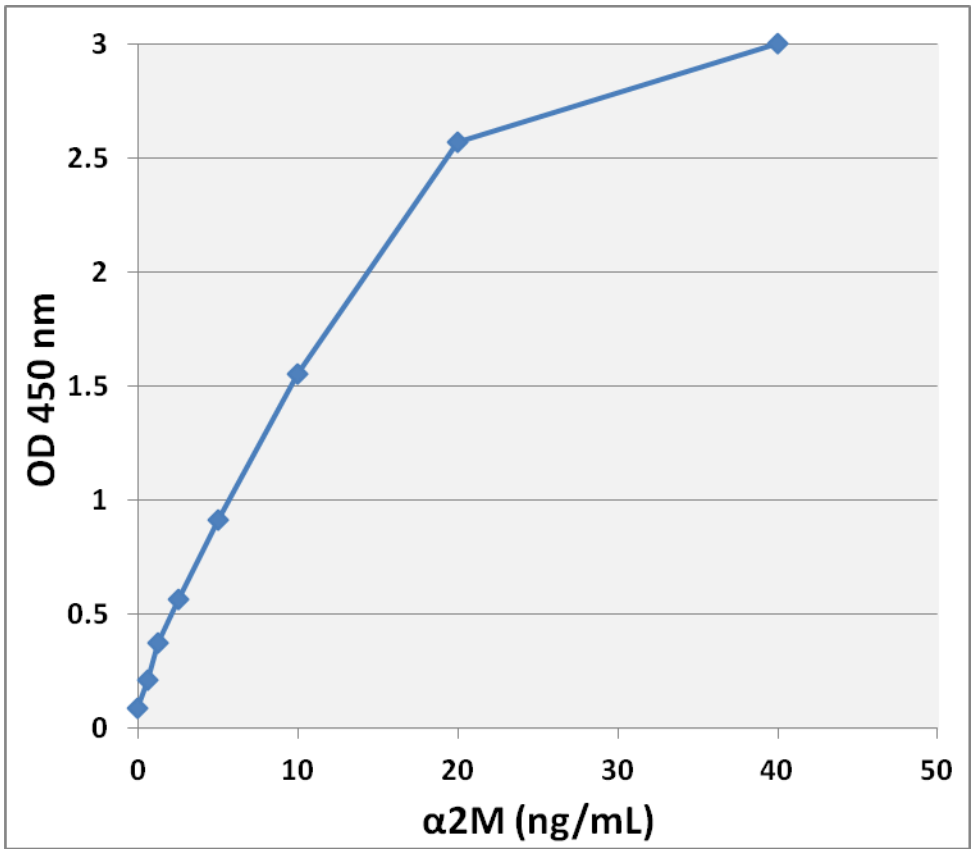

Figure 1: Human α2M ELISA Standard Curve.

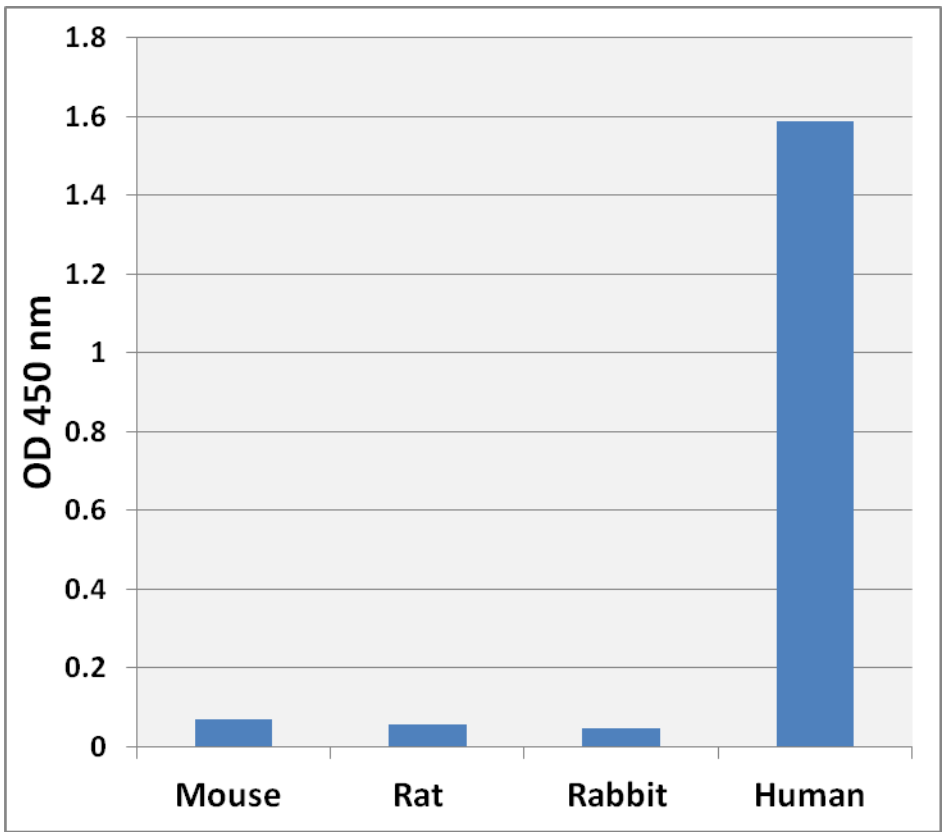

Figure 2: Detection of α2M in serum. Each serum sample was diluted 10,000 fold according to the protocol above and then tested using the Human α2M ELISA Kit.

References

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4. Weaver, A. M., Owens, G. K., and Gonias, S. L. (1995) *J. Biol. Chem.* **270**: 30741–30748
5. Philip, A., and O'Connor-McCourt, M. D. (1991) *J. Biol. Chem.* **266**: 22290–22296

Warranty

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