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Product Manual

# Human Alpha 1 Antitrypsin ELISA Kit

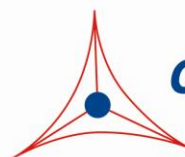
Catalog Number

PRB-5034

96 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **Introduction**

Alpha-1 Antitrypsin (A1AT) is a protease inhibitor that belongs to the serine protease inhibitor (serpin) superfamily. Although originally characterized as a serum trypsin inhibitor, A1AT also inhibits a wide range of proteases. A1AT protects tissues from damage caused by enzymes such as neutrophil elastase which is expressed by inflammatory cells. If A1AT is not expressed or is inactive due to mutation, neutrophil elastase breaks down elastin which leads to increased lung elasticity and respiratory problems such as emphysema, chronic obstructive pulmonary disease (COPD), and cirrhosis of the liver. A1AT deficiency has also been linked to hypercholesterolemia, asthma, granulomatosis with polyangiitis, pancreatitis, gallstones, bronchiectasis and pelvic organ prolapse.

Cell Biolabs' Human Alpha 1 Antitrypsin ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human A1AT in plasma, serum, cell or tissue lysate samples. The kit has a detection sensitivity limit of 300 ng/mL human A1AT. 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. STA-214: Copper (Cu<sup>++</sup>) Oxidized Human Low Density Lipoprotein (LDL)
3. STA-368: Human ApoB ELISA Kit
4. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
5. STA-385: PCSK9 ELISA Kit
6. STA-386: Human LDLR ELISA Kit
7. STA-387: Human LOX-1 ELISA Kit
8. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
9. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

## **Kit Components**

### **Box 1 (shipped at room temperature)**

1. Anti-Human A1AT Antibody Coated Plate (Part No. 50341B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Human A1AT Antibody (1000X) (Part No. 50342C): One 10 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 A1AT Standard (Part No. 50343D): One 50  $\mu$ L vial of 1 mg/mL Human A1AT.

### **Materials Not Supplied**

1. Plasma, serum, cell or tissue lysate
2. PBS containing 0.1% BSA
3. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
4. 50  $\mu$ L to 300  $\mu$ L 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 A1AT Standard at  $-80^{\circ}\text{C}$  to avoid multiple freeze/thaw cycles. Store all other components at  $4^{\circ}\text{C}$ .

### **Preparation of Reagents**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Human A1AT Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human A1AT antibody and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

### **Preparation of Human A1AT Standard**

Prepare a dilution series of human A1AT standards in the concentration range of 0 to 20  $\mu\text{g/mL}$  into Assay Diluent (Table 1).

<b>Standard Tubes</b>	<b>1 mg/mL Human A1AT Standard (<math>\mu\text{L}</math>)</b>	<b>Assay Diluent (<math>\mu\text{L}</math>)</b>	<b>Human A1AT (<math>\mu\text{g/mL}</math>)</b>
1	10	490	20
2	250 of Tube #1	250	10
3	250 of Tube #2	250	5
4	250 of Tube #3	250	2.5
5	250 of Tube #4	250	1.25
6	250 of Tube #5	250	0.625
7	250 of Tube #6	250	0.313
8	0	250	0

**Table 1. Preparation of Human A1AT 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 -80°C for up to three months. Normal plasma samples require 1:50-1:400 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- **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 -80°C for up to three months. Normal serum samples require 1:50-1:400 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- **Urine:** Harvest urine and centrifuge for 10 minutes at 1000 g at 4°C. Assay immediately or store samples at -80°C 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 4°C. Assay immediately or store samples at -80°C 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 containing 1% NP40 and centrifuge at 10,000 x g for 10 minutes at 4°C. Assay immediately or store samples at -80°C for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

## **Assay Protocol**

1. Add 100 µL of human A1AT unknown sample or standard to the Anti-Human A1AT Antibody Coated Plate. Each human A1AT 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 A1AT 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.

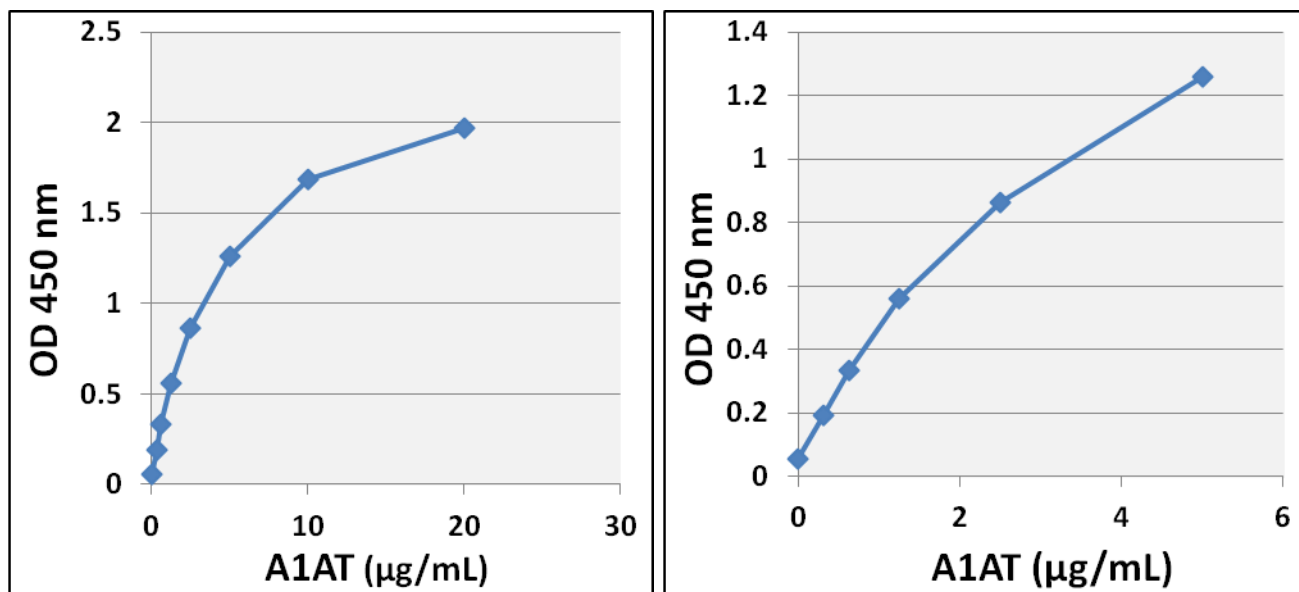
- Warm Substrate Solution to room temperature. Add 100  $\mu\text{L}$  of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

*Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.*

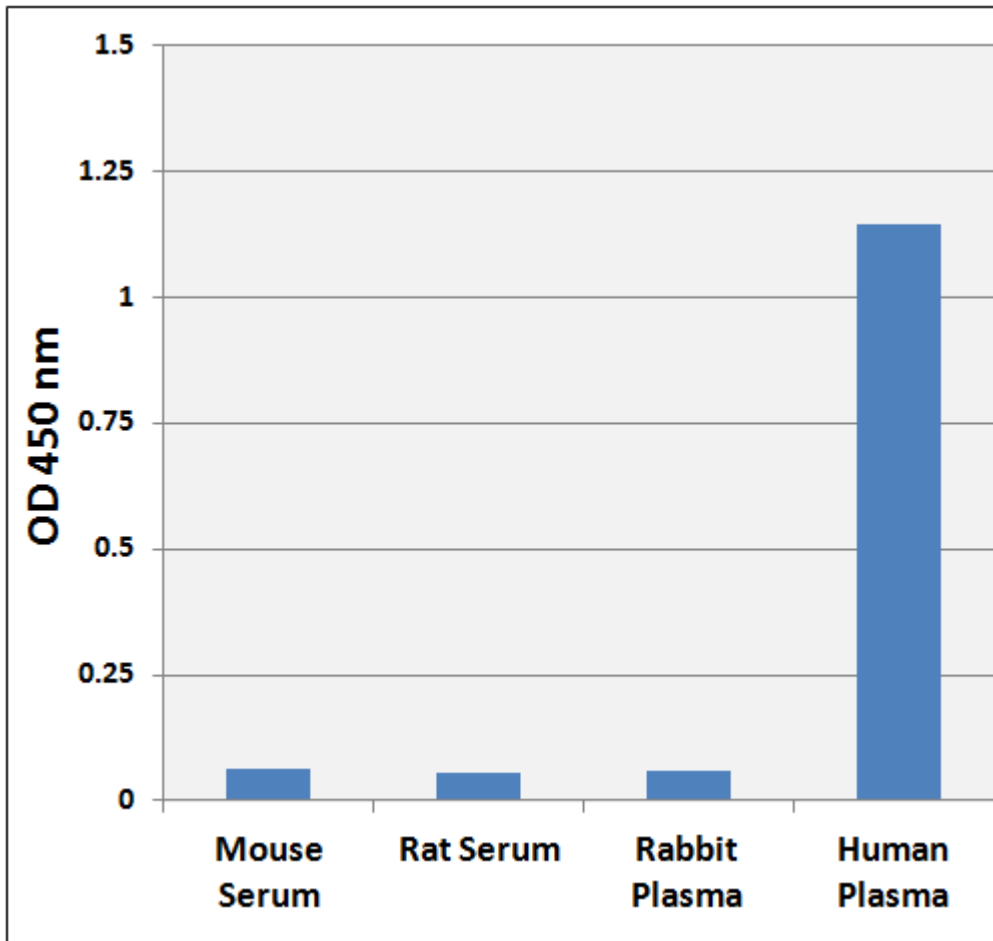
- Stop the enzyme reaction by adding 100  $\mu\text{L}$  of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
- Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

### **Example of Results**

The following figures demonstrate typical results with the Human Alpha 1 Antitrypsin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: Human Alpha 1 Antitrypsin ELISA Standard Curve.**



**Figure 2: Detection of Alpha 1 Antitrypsin in plasma or serum.** Each serum or plasma sample was diluted 400 fold according to the protocol above and then tested using the Human Alpha 1 Antitrypsin ELISA Kit.

### **References**

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2. DeMeo DL, and Silverman, EK (2004) Thorax 59: 259–264.
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7. Diliberti, JH et al (1984) Am. J. Med. Sci. 288:81-85.

### **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

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