ab108798

alpha 1 Antitrypsin

Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human alpha 1 Antitrypsin concentrations in plasma and serum

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Alpha 1 antitrypsin (A1AT) is a protein that protects the lungs. The liver usually makes the protein, and releases it into the bloodstream. alpha 1 Antitrypsin is a major protease inhibitor that controls tissue degradation. A reduction of alpha 1 Antitrypsin levels can cause a change in collagen metabolism. alpha 1 Antitrypsin inhibits neutrophil elastase release into the lungs during inflammatory states. alpha 1 Antitrypsin deficiency is an uncommon genetic disease that can lead to emphysema, hepatitis, cirrhosis, chronic obstructive pulmonary disease (COPD).

ab108798 alpha 1 Antitrypsin Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human alpha 1 Antitrypsin in plasma, and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures human alpha 1 Antitrypsin in less than 3 hours. A polyclonal antibody specific for human alpha 1 Antitrypsin has been pre-coated onto a 96-well microplate with removable strips. alpha 1 Antitrypsin in standards and samples is competed by a biotinylated alpha 1 Antitrypsin sandwiched by the immobilized antibody and 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.
2. Assay Summary

Prepare all reagents, samples and standards as instructed.

\[ \downarrow \]

Add 25 \( \mu l \) standard or sample to each well and immediately add

25 \( \mu l \) of Biotinylated alpha 1 Antitrypsin to each well.

Incubate 2 hours at room temperature.

\[ \downarrow \]

Wash the microplate 5 x with wash buffer.

\[ \downarrow \]

Add 50 \( \mu l \) of Streptavidin-Peroxidase Conjugate.

Incubate 30 minutes at room temperature.

\[ \downarrow \]

Wash the microplate 5 x with wash buffer.

\[ \downarrow \]

Add 50 \( \mu l \) of Chromogen Substrate to each well.

Incubate 10 minutes or till the optimal blue colour density develops.

\[ \downarrow \]

Add 50 \( \mu l \) Stop Solution to each well. Read at 450 nm immediately.
3. Kit Contents

- Human alpha 1 Antitrypsin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human alpha 1 Antitrypsin.
- Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- Human alpha 1 Antitrypsin Standard: Human alpha 1 Antitrypsin in a buffered protein base.
- Biotinylated alpha 1 Antitrypsin: 1 vial, lyophilized.
- Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml).
- Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml).
- 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).
4. Storage and Handling

ab108798 may be stored until the expiration date at 2 to 8°C or -20°C from the date of shipment. Opened diluents may be stored for up to 1 month at 2 to 8°C. Store reconstituted reagents at -20°C or below. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 450nm.
- Precision pipettes to deliver 1 μl to 1 ml volumes.
- Distilled or deionized reagent grade water.
6. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures

- Do not use kit or components if it has exceeded the expiration date on the kit labels

- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
7. Technical Hints

• It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time.

• The coefficient of determination of the standard curve should be $\geq 0.95$ and the highest O.D. should be more than 1.0.

• Cover or cap all kit components and store at 2-8° C when not in use.

• Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity.

• Samples should be collected in pyrogen/endotoxin-free tubes.

• When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.

• When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
• Do not mix or interchange different reagent lots from various kit lots.

• Read absorbance immediately after adding the stop solution.

• Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.

• Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.
8. Preparation of Reagents

Sample Collection:

1. **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 1:800 into 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.)

2. **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 1:800 into Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

1. Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, gently until the crystals have completely dissolved.

2. **Diluent Concentrate (10x):** Dilute the Diluent 1:10 with reagent grade water. Store for up to 1 month at 2 to 8°C.

3. **Standard Curve:** Reconstitute the alpha 1 Antitrypsin Standard with the appropriate amount of Diluent to generate a solution of 40 μg/ml. Allow the standard to sit for 10 minutes with gentle
agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (40 μg/ml) 1:4 with Diluent to produce 10, 2.5, 0.625, 0.156 and 0.039 μg/ml solutions. Diluent serves as the zero standard (0 μg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

<table>
<thead>
<tr>
<th>Standard Point</th>
<th>Dilution</th>
<th>[α1 antitrypsin] (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Standard (40 μg/ml)</td>
<td>40.000</td>
</tr>
<tr>
<td>P2</td>
<td>1 part P1 + 3 parts Diluent</td>
<td>10.000</td>
</tr>
<tr>
<td>P3</td>
<td>1 part P2 + 3 parts Diluent</td>
<td>2.500</td>
</tr>
<tr>
<td>P4</td>
<td>1 part P3 + 3 parts Diluent</td>
<td>0.625</td>
</tr>
<tr>
<td>P5</td>
<td>1 part P4 + 3 parts Diluent</td>
<td>0.156</td>
</tr>
<tr>
<td>P6</td>
<td>1 part P5 + 3 parts Diluent</td>
<td>0.039</td>
</tr>
<tr>
<td>P7</td>
<td>Diluent</td>
<td>0.000</td>
</tr>
</tbody>
</table>

4. **Biotinylated α1 Antitrypsin (2x):** Dilute Biotinylated α1 Antitrypsin with 4 ml Diluent to produce 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilution. The stock solution should be further diluted 1:2 with Diluent. Any remaining solution should be frozen at -20°C and used within 30 days.

5. **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
6. **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent. Any remaining solution should be frozen at -20°C.

9. **Assay Method**

7. 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).

8. 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.

9. Add 25 μl of standard or sample per well and immediately add 25 μl of Biotinylated alpha 1 Antitrypsin to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

10. Wash five times with 200 μl of Wash Buffer. Invert the plate and decant the contents, and tap it 4-5 times on absorbent paper towel to completely remove liquid at each step.

11. Add 50 μl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

12. Wash five times with 200 μl of Wash Buffer.
13. Add 50 μl of Chromogen Substrate per well and incubate for about 10 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.

14. Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.

15. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.
10. Data Analysis

Calculate the mean value of the 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.

A. Typical Data

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

B. Sensitivity

The minimum detectable dose of alpha 1 Antitrypsin is typically 40 ng/ml.
C. Recovery

Standard Added Value: 0.5 – 10 μg/ml

Recovery %: 87-111

Average Recovery %: 98

D. Reproducibility

Intra-Assay: CV = 4.5%

Inter-Assay: CV = 7.2%

E. Linearity

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Average % of Expected Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
</tr>
<tr>
<td>1:400</td>
<td>104</td>
</tr>
<tr>
<td>1:800</td>
<td>100</td>
</tr>
<tr>
<td>1:1600</td>
<td>96</td>
</tr>
</tbody>
</table>
11. Specificity

No significant cross-reactivity or interference was observed.

<table>
<thead>
<tr>
<th>Species</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Mouse</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Rat</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Swine</td>
<td>None</td>
</tr>
<tr>
<td>Beagle</td>
<td>None</td>
</tr>
<tr>
<td>Bovine</td>
<td>None</td>
</tr>
<tr>
<td>Rabbit</td>
<td>None</td>
</tr>
</tbody>
</table>

Note: The normal blood levels of alpha 1 Antitrypsin are 1.5 -3.5 g/l.
## 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; Concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
UK, EU and ROW
Email: technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 108008523689 (中國聯通)
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp

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