

Human Alpha-1 antitrypsin (A1AT) Immunoassay kit

Catalogue Number: 31420

For the quantitative determination of human Alpha-1 antitrypsin
concentrations in serum and plasma samples

This package insert must be read in its entirety before using this product.

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INTRODUCTION

Alpha-1 antitrypsin (A1AT), also known as alpha-1 protease inhibitor or SERPINA1, is a 52 kDa serine protease inhibitor, which is mainly synthesized in the liver, but also produced in monocytes, macrophages, dendritic cells, pulmonary alveolar cells, intestinal and corneal epithelium [1]. The primary function of A1AT is to inhibit the actions of proteolytic enzymes, such as neutrophil elastase (NE), proteinase 3 (PR3) and cathepsin G (CG), and to provide essential protection to host tissues from non-specific injury during periods of inflammation [2]. A1AT has also been reported to regulate neutrophil chemotaxis, to enhance insulin secretion and protect β -cells against cytokine-induced apoptosis, and to possess the anti-inflammatory as well as immunomodulatory properties [3-5]. Systemic deficiency of A1AT due to genetic mutations can result in a number of diseases, such as chronic obstructive pulmonary disease (COPD), systemic sclerosis, liver injury, cirrhosis and hepatocellular carcinoma [6-8]. Furthermore, A1AT disorder has also been shown to be involved in the development of diabetes mellitus [9, 10].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich ELISA. The immunoplate is pre-coated with a polyclonal antibody specific for human A1AT. Standards and samples are pipetted into the wells and any human A1AT present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked polyclonal antibody specific for human A1AT is added to the wells. After a final wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human A1AT bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human A1AT, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This human A1AT ELISA kit is designed for quantification of human A1AT in serum and plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)-Coated with a polyclonal antibody against human A1AT, sealed.
2. 10×Wash buffer-40 ml.
3. 5×Assay buffer-60 ml.
4. 100×Detection antibody solution-A HRP labelled polyclonal antibody against human A1AT, 0.12 ml.
5. Human A1AT standard-20 ng of native human A1AT in a buffered protein base, lyophilised.
6. Substrate solution- 12 ml, ready for use.
7. Stop solution- 12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human A1AT microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer.

Prepare 1×Assay buffer by mixing the 5×Assay buffer (60 ml) with 240 ml of distilled water or deionized water. If precipitates are observed in the 5× Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 ml) with 360 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDRADS, CONTROL AND SAMPLE

Human A1AT standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 20 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
20 ng/ml stock	-	20 ng/ml
250 µl of 20 ng/ml	250 µl	10 ng/ml
250 µl of 10 ng/ml	250 µl	5 ng/ml
250 µl of 5 ng/ml	250 µl	2.5 ng/ml
250 µl of 2.5 ng/ml	250 µl	1.25 ng/ml
250 µl of 1.25ng/ml	250 µl	0.625ng/ml
250 µl of 0.625 ng/ml	250 µl	0.312 ng/ml

1×Assay buffer serves as the zero standard (0 pg/ml).

Note: The reconstituted standard stock should be aliquoted and stored at -80°C for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required a 1000000-fold dilution in this assay. A three-step dilution is suggested. Step1- Add 10 µl of sample to 990 µl of 1× Assay buffer to give a 100-fold diluted sample solution. Step 2- Add 5 µl of the 100-fold diluted sample solution to 495 µl of 1×Assay buffer to give a 10000-fold diluted sample solution. Step 3- Add 5 µl of the

10000-fold diluted sample solution to 495 μ l of 1 \times Assay buffer to give a final 1000000-fold diluted sample solution. If a sample has A1AT level greater than the highest standard, the sample should be diluted further and the assay should be repeated.

ASSAY PROCEDURE

It is recommended that all standards, control and samples should be assayed in duplicate.

1. Add 100 μ l of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 μ l of 1 \times Wash buffer to each well and incubate for 1 minute. Discard the 1 \times Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 μ l of 1 \times Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 μ l of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 μ l of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

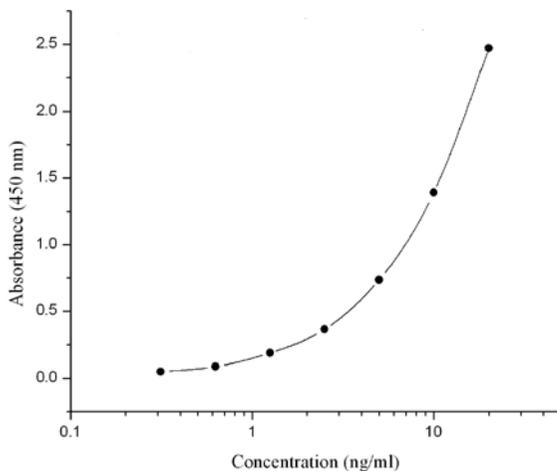
1. Subtract absorbance of blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human A1AT concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human A1AT concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Human A1AT (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.08	0
0.312	0.129	0.049
0.625	0.166	0.086
1.25	0.267	0.187
2.5	0.445	0.365
5	0.816	0.736
10	1.47	1.39
20	2.55	2.47

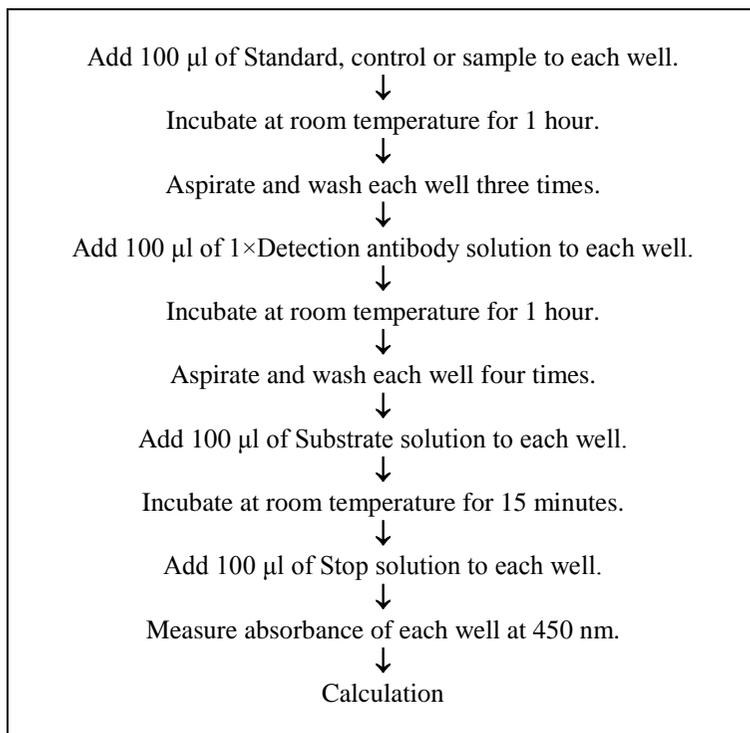
Human A1AT standard curve (4-parameter)



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SUMMARY OF ASSAY PROCEDURE



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