ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human Alpha-1 Antitrypsin (A1AT) ELISA Kit

(Catalog Number: 31390)

For the quantitative determination of human alpha-1 antitrypsin concentrations in serum or plasma

IMD (Hong Kong)
Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)
Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

Version 2.0

TABLE OF CONTENTS

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 enzyme-linked immunosorbent assay (ELISA). The microtiter plate 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 3,3′,5,5′-Tetramethylbenzidine (TMB) is added and color develops in proportion to the amount of human A1AT bound initially. The assay is stopped, and the optical density of the wells is 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. Microtiter 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, an HRP labelled polyclonal antibody against human A1AT, 0.12 mL
5. Human A1AT standard, 20 ng of native human A1AT, lyophilized
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 microtiter plate, 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

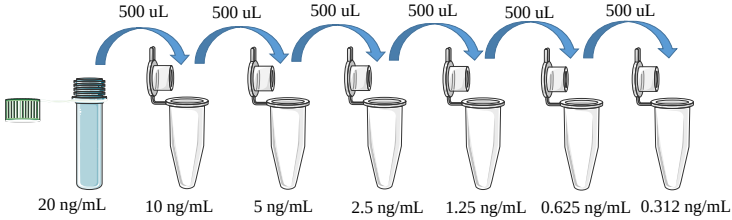
Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

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 STANDARDS, CONTROLS AND SAMPLES

Human A1AT Standards: Reconstitute the lyophilized 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. Pipette 500 µL of 1×Assay buffer to 10, 5, 2.5, 1.25, 0.625, 0.312 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 pg/mL). The reconstituted standard stock should be aliquoted and stored at -80ºC for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires 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 µL of 1×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. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

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

1. Add 100 µL 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 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×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 µL of 1×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 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light**.
6. Add 100 µL 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.

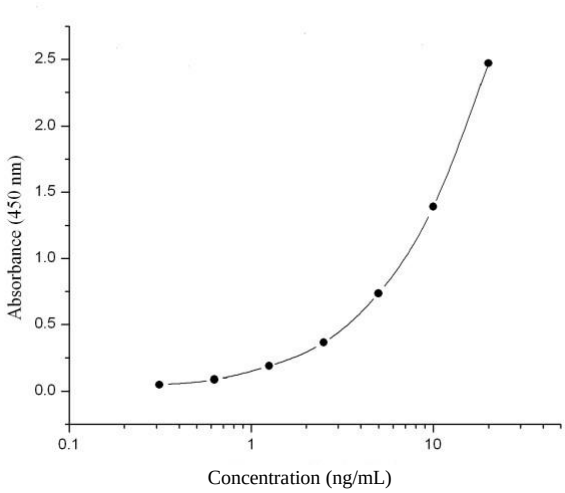
3. Determine human A1AT concentration of samples from standard curve and multiply the value by the dilution factor.

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)



REFERENCES

1. Janciauskiene SM, Bals R, Koczulla R, Vogelmeier C, Kohnlein T, Welte T: The discovery of alpha1-antitrypsin and its role in health and disease. *Respir Med* 2011, 105:1129-1139.
2. Miravitlles M: Alpha-1-antitrypsin and other proteinase inhibitors. *Curr Opin Pharmacol* 2012, 12:309-314.
3. Bergin DA, Reeves EP, Meleady P, Henry M, McElvaney OJ, Carroll TP,
4. Condron C, Chotirmall SH, Clynes M, O'Neill SJ, McElvaney NG: alpha1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. *J Clin Invest* 2010, 120:4236-4250.
5. Kalis M, Kumar R, Janciauskiene S, Salehi A, Cilio CM: alpha 1antitrypsin enhances insulin secretion and prevents cytokine-mediated apoptosis in pancreatic beta-cells. *Islets* 2010, 2:185-189.
6. Jonigk D, Al-Omari M, Maegel L, Muller M, Izykowski N, Hong J, Hong K, Kim SH, Dorsch M, Mahadeva R, et al: Anti-inflammatory and immunomodulatory properties of alpha1-antitrypsin without inhibition of elastase. *Proc Natl Acad Sci U S A* 2013, 110:15007-15012.
7. Brebner JA, Stockley RA: Recent advances in alpha-1-antitrypsin deficiency-related lung disease. *Expert Rev Respir Med* 2013, 7:213-229; quiz 230.
8. Barnes TC, Cross A, Anderson ME, Edwards SW, Moots RJ: Relative alpha(1)-anti-trypsin deficiency in systemic sclerosis. *Rheumatology (Oxford)* 2011, 50:1373-1378.
9. Teckman JH: Liver disease in alpha-1 antitrypsin deficiency: current understanding and future therapy. *COPD* 2013, 10 Suppl 1:35-43.
10. Mansuy-Aubert V, Zhou QL, Xie X, Gong Z, Huang JY, Khan AR, Aubert G, Candelaria K, Thomas S, Shin DJ, et al: Imbalance between neutrophil elastase and its inhibitor alpha1-antitrypsin in obesity alters insulin sensitivity, inflammation, and energy expenditure. *Cell Metab* 2013, 17:534-548.
11. Wang Y, Xiao Y, Zhong L, Ye D, Zhang J, Tu Y, Bornstein SR, Zhou Z,
12. Lam KS, Xu A: Increased Neutrophil Elastase and Proteinase 3 and Augmented NETosis Are Closely Associated with beta-cell Autoimmunity in Patients with Type 1 Diabetes. *Diabetes* 2014.

SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard, control or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation