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Human Alpha-1-Antitrypsin Total Antigen ELISA Kit

Catalog # HA1ATKT-TOT

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

This human alpha-1-antitrypsin (A1AT) assay is for the quantitative determination of total A1AT in human plasma, serum, saliva, and urine. **For research use only.**

BACKGROUND

A1AT, also known as alpha-1 protease inhibitor (A1PI), is a glycosylated 394 amino acid 52 kDa plasma serpin that is the most abundant proteinase inhibitor in plasma [1]. Although it does inhibit trypsin and similar proteinases, its physiological target is neutrophil elastase. Individuals with genetically low expression of A1AT are at risk for tissue and organ damage due to unchecked neutrophil elastase activities [2]. A1AT deficiency is associated with respiratory complications such as chronic obstructive pulmonary disease [3]. Mutations in A1AT can lead to non-functional proteins that polymerize and accumulate in the liver as in infantile hepatic cirrhosis [4]. These conditions are life threatening and require regular injections of purified A1AT from human plasma [5].

ASSAY PRINCIPLE

Human A1AT will bind to the affinity purified capture antibody coated on the microtiter plate. Complexed and free A1AT will react with the antibody on the plate. After appropriate washing steps, biotinylated polyclonal anti-human A1AT primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total A1AT present in the samples, is reacted with horseradish peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human A1AT. Color development is proportional to the concentration of A1AT in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human A1AT antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human A1AT standard:** 1 vial lyophilized standard
- **Anti-human A1AT primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard` and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using citrate, EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 200ng/ml standard solution.

Dilution table for preparation of human A1AT standard:

A1AT concentration (ng/ml)	Dilutions
100	500µl (BB) + 500µl (from vial)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0. 5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl A1AT standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures A1AT antigen in the 0.1-100 ng/ml range. If the unknown is thought to have high A1AT levels, dilutions may be made in blocking buffer. A 1:200,000 to 1:2,000,000 dilution for normal human plasma and serum samples, 1:10 dilution for urine samples, and no dilution for saliva is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 1: 50,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

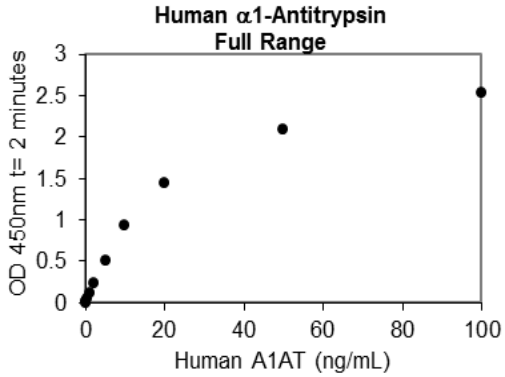
Measurement

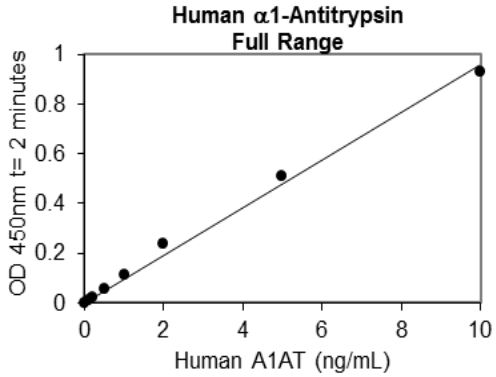
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of α2-antiplasmin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

The average concentration of A1AT in normal human plasma and serum is 1.3 mg/ml [6].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.047-0.055) and calculating the corresponding concentration. The MDD was 0.062ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.314	3.2	58.6
Standard Deviation	0.023	0.093	4.31
CV (%)	7.39	2.9	7.34

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.339	3.34	62
Standard Deviation	0.016	0.297	7.49
CV (%)	4.63	8.77	12.1

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.26	2.46	11.2	26.4
Average % Recovery	102	98	93	106
Range	98-108%	97-99%	91-97%	98-109%

Linearity: To assess the linearity of the assay, human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of expected	100	101	104	103
Range	97-103%	97-103%	101-107%	102-106%

Specificity: Pooled normal plasma or serum from mouse, rat, pig, horse, rabbit, sheep, hamster, and canine were assayed for cross-reactivity. No significant cross-reactivity was observed.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:400,000	2230
	1:1,600,000	2210
EDTA Plasma	1:400,000	2210
Heparin Plasma	1:400,000	2530
Serum	1:400,000	2300
Urine	1:10	0.28
Saliva	Undiluted	0.035

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Donato LJ, *et al.*: Am J Clin Pathol. 2012, 138: 398-405.
2. Stoller JK & Aboussouan LS: Lancet. 2005, 365:2225-2236.
3. Janciauskiene SM, *et al.*: Respir Med. 2011, 105:1129-1139.
4. Teckman JH: COPD. 2013, 10 Suppl 1:35-43.
5. Brantly ML, *et al.*: COPD. 2019, 6:100-114.
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Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
B	0	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
C												
D												
E												
F												
G												
H												