

LSBio™ Human SERPINC1 / Antithrombin III ELISA Kit

Catalog No. LS-F10410

User Manual

**Please Read the Manual Carefully
Before Starting your Experiment**



For research use only. Not approved for use in humans or for clinical diagnosis.



LifeSpan BioSciences, Inc.

www.lsbio.com

Human Antithrombin III Total Antigen ELISA Kit

Catalog # LS-F10410 Strip
well format. Reagents for up to 96 tests.

Rev: November 2014

INTENDED USE

This human Antithrombin III antigen assay is intended for the quantitative determination of total Antithrombin III antigen in human plasma.

For research use only.

BACKGROUND

Antithrombin III is a glycosylated plasma serine protease inhibitor that forms a stoichiometric complex with coagulation cascade enzymes [1]. Antithrombin III inhibits alpha-Thrombin as well as Factor Xa, IXa, XIa and XIIa with heparin enhanced kinetics [2]. Type 1 Antithrombin deficiency is characterized by decreased plasma antigen levels of Antithrombin III [3].

ASSAY PRINCIPLE

Human Antithrombin III will bind to the capture antibody coated on the microtiter plate. Free and complexed Antithrombin III will react with the antibody on the plate. After appropriate washing steps, polyclonal anti-human Antithrombin III primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total Antithrombin III present in the samples, reacts with the secondary antibody. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared using dilutions of purified Antithrombin III and is measured along with the test samples. Color development is proportional to the concentration of Antithrombin III in the samples.

STANDARD CALIBRATION

Antithrombin III standard provided is calibrated against the WHO 3rd International Standard for Antithrombin, Plasma distributed by NIBSC (08/258), South Mimms, Potters Bar, Hertfordshire, UK.

REAGENTS PROVIDED

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

STORAGE AND STABILITY

HRP conjugated secondary antibody must be stored at $\leq -70^{\circ}\text{C}$. Store all other kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards 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 EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. 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 7ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 50ng/ml standard solution.

Dilution table for preparation of human antithrombin III standard:

Antithrombin III concentration (ng/ml)	Dilutions
20	600 μl (BB) + 400 μl (std vial)
10	500 μl (BB) + 500 μl (20ng/ml)
5	500 μl (BB) + 500 μl (10ng/ml)
2	600 μl (BB) + 400 μl (5ng/ml)
1	500 μl (BB) + 500 μl (2ng/ml)
0.5	500 μl (BB) + 500 μl (1ng/ml)
0.2	600 μl (BB) + 400 μl (0.5ng/ml)
0.1	500 μl (BB) + 500 μl (0.2ng/ml)
0.05	500 μl (BB) + 500 μl (0.1ng/ml)
0.02	600 μl (BB) + 400 μl (0.05ng/ml)
0	500 μl (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 100 μl antithrombin III 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 300 μl wash buffer. Remove

excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures antithrombin III antigen in the 0.02-20 ng/ml range. If the unknown is thought to have high antithrombin levels, dilutions may be made in blocking buffer. A 1:100,000-1:400,000 dilution for normal human plasma 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 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2 μl of conjugated secondary antibody in 10ml of blocking buffer and add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl 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 2-15 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H₂SO₄ 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}).

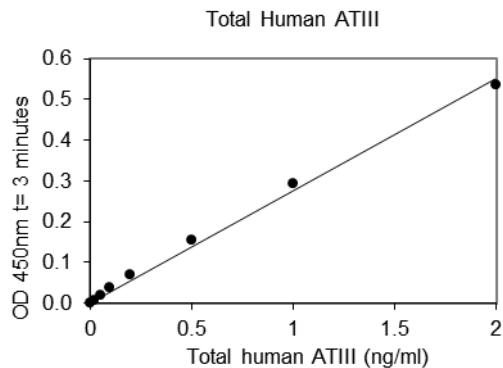
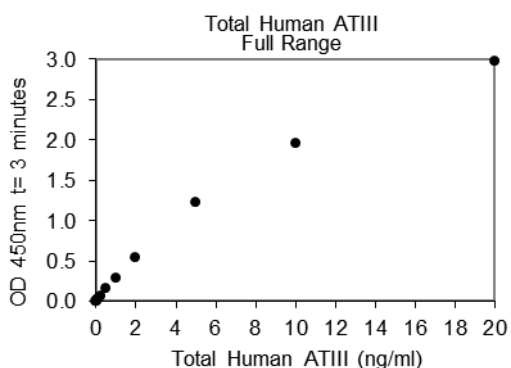
Calculation of Results

Plot A_{450} against the amount of antithrombin III 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 antithrombin III 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 concentration of Antithrombin III in normal human plasma by thrombin titration is 2.57 µM or 150 µg/ml [4]. An antigen concentration of 137 µg/ml in human reference plasma was determined by house testing at 1:100,000 and 1:200,000 dilutions.

PERFORMANCE CHARACTERISTICS

Sensitivity: These studies are currently in progress. Please contact us for more information.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

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. Rosenberg RD and Damus PS.: J. Biol. Chem. 1973, 248: 6490-6505.
2. Olson ST, *et al.*: Methods in Enzymology. 1993, 222: 525-559.
3. Blajchman MA, *et al.* Blood, 1992, 80: 2159-2171.
4. Conard J, *et al.* Haemostasis, 1983, 13(6): 363-368.

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	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
B	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
C												
D												
E F												
G												
H												

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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