Datasheet for ABIN612655

SERPINC1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	SERPINC1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	3.125-200 ng/mL
Minimum Detection Limit:	3.125 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Antithrombin III ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human ATIII in urine, milk, saliva, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures ATIII in approximately 4 hours. A monoclonal antibody specific for human ATIII has been pre-coated onto a 96- well microplate with removable strips. ATIII in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for human ATIII which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Cerebrospinal Fluid, Milk, Saliva, Urine
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human Antithrombin III Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human ATIII. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay. Human Antithrombin III Standard: Human ATIII in a buffered protein base (400 ng, lyophilized). Biotinylated Human Antithrombin III Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against ATIII (120 l). MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP conjugate): A 100-fold concentrate (80 l). 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).
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water. Incubator (37 °C)

Target Details

Target:	SERPINC1
Alternative Name:	Antithrombin III (AT3) (SERPINC1 Products)
Background:	The serine protease inhibitor antithrombin III (ATIII), the most important natural inhibitor of thrombin activity, has been shown to exert marked anti- inflammatory properties (1). ATIII levels are positively correlated with plasma total cholesterol levels, plasma low-density lipoprotein cholesterol levels, plasma triglycerides, and D-dimer levels (2).
Gene ID:	462
UniProt:	P01008

Application Details

Sample Volume:	50 µL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours. • Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour. • Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.

- Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 12 minutes.
- Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8 °C. Human Antithrombin III Standard: Reconstitute the Human Antithrombin III Standard (400 ng) with 2 mL of MIX Diluent to generate a 200 ng/mL standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (200 ng/mL) 2-fold with equal volume of MIX Diluent to produce 100, 50, 25, 12.5, 6.25, and 3.125 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be frozen at -20 °C and used within 30 days. Avoid repeated freeze-thaw cycles. Standard Point Dilution ATIII (ng/mL) ATIII (mIU/mL) P1 1 part Standard (200 ng/mL) 200 0.3 P2 1 part P1 + 1 part MIX Diluent 100 0.15 P3 1 part P2 + 1 part MIX Diluent 50 0.075 P4 1 part P3 + 1 part MIX Diluent 25 0.038 P5 1 part P4 + 1 part MIX Diluent 12.5 0.019 P6 1 part P5 + 1 part MIX Diluent 6.25 0.009 P7 1 part P6 + 1 part MIX Diluent 3.125 0.005 P8 MIX Diluent 0.0 0.0 Biotinylated Human Antithrombin III Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent. The undiluted antibody should be stored at -20 °C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water. Streptavidin-Peroxidase Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent. The undiluted conjugate should be stored at -20 °C. 5
Sample Collection:	Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 2-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 10-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 200-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. A

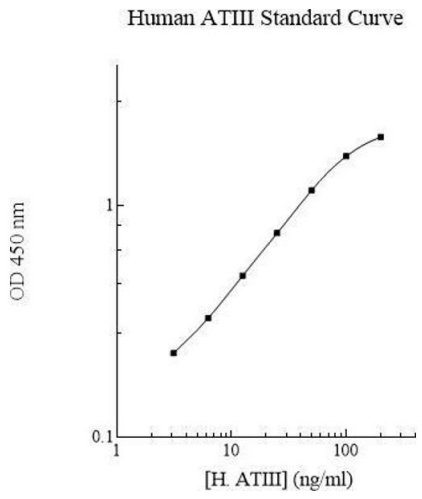
	50-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80 °C for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes at 4 °C to remove debris and collect supernatants. Samples can be stored at -20 °C or below. Avoid repeated freeze-thaw cycles. Refer to Sample Dilution Guidelines for further instruction. Guidelines for Dilutions of 100-fold or Greater (for reference only, please follow the insert for specific dilution suggested) 100x 10000x A) 4 µL sample: 396 µL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL. A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL. 1000x 100000x A) 4 µL sample : 396 µL buffer (100x) B) 24 µL of A : 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL. A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) C) 24 µL of B : 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL. 4
Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Human Antithrombin III Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated Human Antithrombin III Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour. Wash the microplate as described above. Add 50 l of Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 l of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 12 minutes or until the optimal blue color density develops. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that

Application Details

	may have formed. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings. 6
Calculation of Results:	• Calculate the mean value of the duplicate or 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 (OD) 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.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.7 % and 7.3% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	This product is for Research Use Only and is not intended for use in diagnostic procedures. Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents. The Stop Solution is an acidic solution. The kit should not be used beyond the expiration date. Human Antithrombin III (ATIII) ELISA Kit Catalog No. EA3301-1 Sample insert for reference use only 2
Storage:	4 °C/-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.



ELISA
Image 1.

