Datasheet for ABIN5564557

APOC1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	APOC1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.015-1 µg/mL
Minimum Detection Limit:	0.015 µg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human Apolipoprotein C-I ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human ApoC-I in plasma, serum, cell lysates, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human ApoC-I in approximately 5 hours. A polyclonal antibody specific for human ApoC-I has been pre-coated onto a 96-well microplate with removable strips. ApoC-I in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for ApoC-I, 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, Plasma, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human ApoC-I Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human ApoC-I. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human ApoC-I Standard: Human ApoC-I in a buffered protein base (1 g, lyophilized, 2 vials, store at -20°C). Biotinylated Human ApoC-I Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against ApoC-I (140 l). EIA 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:	APOC1
Alternative Name:	Apolipoprotein C-I (Apo C1) (APOC1 Products)
Background:	Apolipoprotein C-I (ApoC-I) is a 6.6 kDa apolipoprotein that is expressed primarily in the liver and activated when monocytes differentiate into macrophases. After being synthesized as a precursor with a length of 83 amino acids, ApoC-I is processed to a single chain mature protein of 57 amino acids (1). It circulates in plasma and is a component of VLDL, IDL, and HDL (2, 3). ApoC-I plays important modulatory roles in lipoprotein metabolism. It is an inhibitor of lipoprotein binding to the LDL receptor, LDL receptor-related protein, and VLDL receptor (4, 5). It is the major plasma inhibitor of cholesteryl ester transfer protein and appears to interfere directly with fatty acid uptake (6, 7). ApoC-I causes hypertriglyceridemia by inhibition of the lipoprotein lipase-dependent triglyceride-hydrolysis pathway (8). On the other hand, ApoC-I is an activator of lecithin cholesterol acyl transferase that esterifies cholesterol and produces the formation of the mature HDL (9, 10).
Gene ID:	341
UniProt:	P02654
Pathways:	Apoptosis

Application Details

Assay Time:	5 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 2 hours. • 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 10 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. EIA Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8 °C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 200-fold into EIA Diluent or within the range of 100x to 400x. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. Dilute samples 200-fold into EIA Diluent or within the range of 100x to 400x. Avoid repeated freeze-thaw cycles. Cell Culture Media: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris and collect supernatants. Store samples at -20 °C or below. Avoid repeated freeze-thaw cycles. Cell Lysate: Rinse cell with cold PBS and then scrape the cell into a tube with 5 mL cold PBS with 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4 °C and aspirate supernatant. Re-suspend pellet in ice- cold Lysis Buffer (10 mM Tris, pH 8.0, 130 mM NaCl, 1 % Triton X-100, protease inhibitor cocktail). For every 1 x 10 6 cells, add approximately 100 µL of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4 °C and collect supernatant for assay. Refer to Sample Dilution Guidelines for further instruction. 4 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 µ L 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.

Application Details

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 ApoC-I Standard or sample per well. 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 ApoC-I antibody to each well and incubate for 2 hours. Wash the microplate as described above. Add 50 l of Streptavidin-Peroxidase conjugate to each well 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 per well and incubate for 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. 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 6 after stopping the reaction for about 10 minutes, which will reduce the readings.
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.
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. 2 Prepare all samples prior to running the

Handling

	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.
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, Biotinylated Antibody, and Standard 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. 3