Datasheet for ABIN612666

APOC1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	APOC1
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.06 µg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Apo C-I ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo C-I in plasma, serum and cell culture samples
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Plasma reference value: 40 70 µg/ml.
Cross-Reactivity (Details):	No significant cross-reactivity was observed with human Apo A-I, Apo A-II, Apo B and Apo E.
Components:	Human Apo C-I Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo C-I. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. 1 Human Apo C-I Standard: Human Apo C-I in a buffered protein base (2 µg, lyophilized, 2 vials, Store at 200C) Biotinylated Apo C-I Antibody (100x): A 100-fold concentrated biotinylated

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	polyclonal antibody against Apo C-I (80µl). 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 (80µl). 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 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	APOC1
Alternative Name:	Apolipoprotein C-I (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. 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. 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). It is also a physiological protector against infection by enhancing the early inflammatory response to lipopolysaccharide.
Pathways:	Apoptosis

Application Details

Sample Volume:	50 µL
Assay Time:	< 5 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Apo C-I in less than 5 hours. A polyclonal antibody specific for human Apo C-I has been

	pre-coated onto a 96-well microplate with removable strips. Apo C-I in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Apo C-I, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 2 g of Apo C-I Standard with 0.5 ml of EIA Diluent to generate a solution of 4 g/ml. Allow the standard to sit on ice for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (4 g /ml) 1:2 with EIA Diluent to produce 2, 1, 0.5, 0.25, 0.125 and 0.063 g/ml solutions. EIA Diluent serves as the zero standard (0 g/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Apo C-I] (g/ml) Standard (4 g/ml) P1 4.000 P2 1 part P1 + 1 part EIA Diluent 2.000 P3 1 part P2 + 1 part EIA Diluent 1.000 P4 1 part P3 + 1 part EIA Diluent 0.500 P5 1 part P4 + 1 part EIA Diluent 0.250 P6 1 part P5 + 1 part EIA Diluent 0.125 P7 1 part P6 + 1 part EIA Diluent 0.063 P8 EIA Diluent 0.000 Biotin Apo C-I Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:200 into EIA Diluent. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:200 into EIA Diluent. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of Apo C-I standard or sample per well. Cover wells with a

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	sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated Apo C-I Antibody to each well and incubate for two hours. Wash a microplate as described above. Add 50 µL 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 a microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 20 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 µL 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 after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	Calculate the mean value of the 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 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. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.5% and 7.2% respectively.
Restrictions:	For Research Use only

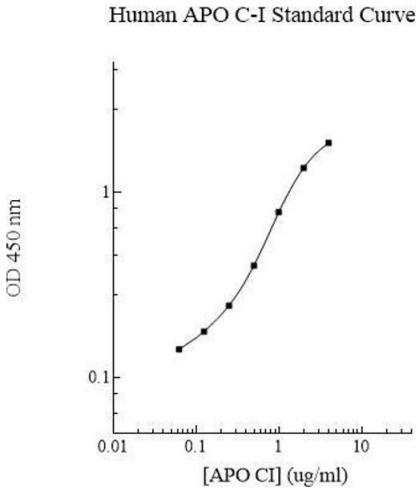
Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened EIA Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Publications

Product cited in:	Yan, Wang, Liu, Zhang, Zhao, Hua, Xu, Li: "A pro-atherogenic HDL profile in coronary heart disease patients: an iTRAQ labelling-based proteomic approach." in: **PLoS ONE**, Vol. 9, Issue 5, pp. e98368, (2014) (PubMed).

Validation report #102978 for ELISA (ELISA)



ELISA

Image 1.