Datasheet for ABIN612661

APOA2 ELISA Kit

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Overview

Quantity:	96 tests
Target:	APOA2
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	50 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Apo A-II ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo A-II in plasma, serum, urine and cell culture supernatant
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	No significant cross reactivity with Apo AI, Apo B, Apo CI, Apo CII, Apo CIII or Apo E.
Components:	Human Apo A-II Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human Apo A-II. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Apo A-II Standard: Human Apo A-II in a buffered protein base (32 µg, lyophilized). 1 Biotinylated Apo A-II Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Apo A-II (80µl). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml).

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	Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (90µl). 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:	APOA2
Alternative Name:	Apolipoprotein A-II (APOA2 Products)
Background:	Apolipoprotein A-II (apoA-II) is the second most abundant apolipoproteins in human plasma HDL, comprising about 25% of the protein mass. After being synthesized by the liver and intestine as a preprotein containing 100 amino acids, apoA-II is processed to 77 amino acids in the mature plasma protein (1 - 3). ApoA-II is found in plasma as a monomer, homodimer of 17.4 kDa, or heterodimer with apoE and apoD (4 - 7). It has been reported that apoA-II plays roles in HDL remodeling, cholesterol efflux, modulating HDL interaction with enzymes and receptors, triglyceride metabolism, and atherosclerosis (7 - 12). ApoA-II is inversely associated with risk of future coronary artery disease. Serum levels of an isoform of apoA-II have been identified as a potential marker for prostate cancer.
Pathways:	Regulation of Lipid Metabolism by PPARalpha, Production of Molecular Mediator of Immune Response, Negative Regulation of Transporter Activity, Lipid Metabolism

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Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Apo A-II in less than 4 hours. A monoclonal antibody specific for human Apo A-II has been pre-coated onto a 96-well microplate with removable strips. Apo A-II in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for human Apo A-II, 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 32 g of Apo A-II Standard with 2 ml of EIA Diluent to generate a solution of 16 g/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the 2 standard solution (16 g/ml) 1:4 with EIA Diluent to produce 4, 1, 0.25, 0.0625 and 0.0156 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 A-II] (g/ml) Standard (16 g/ml) P1 16.000 P2 1 part P1 + 3 part EIA Diluent 4.000 P3 1 part P2 + 3 part EIA Diluent 1.000 P4 1 part P3 + 3 part EIA Diluent 0.250 P5 1 part P4 + 3 part EIA Diluent 0.063 P6 1 part P4 + 3 part EIA Diluent 0.016 P7 EIA Diluent 0.000 Biotinylated Apo A-II 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 use supernatants. Dilute samples 1:1000 with EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. 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. Remove serum and assay. Dilute samples 1:1000 into EIA Diluent. Store serum at -20°C or below. Avoid repeated freeze-thaw cycles Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 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 A-II standard or sample per well. Cover wells with a

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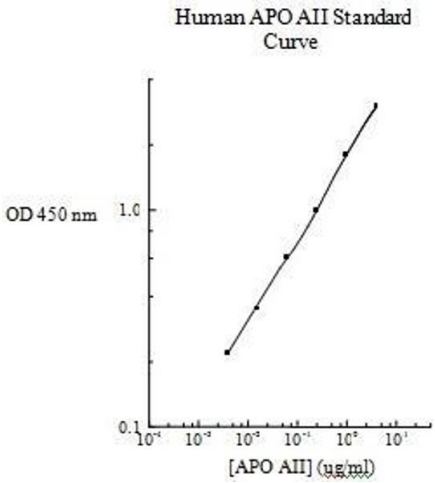
	sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step. Add 50 µL of Biotinylated Apo A-II Antibody to each well and incubate for one hour. Wash five times with 200 µL of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step. 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 five times with 200 µL of Wash Buffer. Add 50 µL of Chromogen Substrate per well and incubate for about 12 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. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings. 3
Calculation of Results:	Calculate the mean value of the duplicate or triplicate readings for each standard, and sample. To generate Standard Curve, plot 4-parameter graph or semi-log graph using the ATIII 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 4.8% and 7.3% 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 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.



ELISA

Image 1.

