Datasheet for ABIN612660

APOA1 ELISA Kit

1 Image | 4 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	APOA1
Reactivity:	Human
Method Type:	Competition ELISA
Minimum Detection Limit:	1.2 µg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Apo A-1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo A-1 in plasma and serum
Brand:	AssayMax
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Reference Value: The normal blood levels of Apo A-1 is ranged from, 130 mg/dL.
Components:	Human Apo A-1 Microplate: A 96-well polystyrene microplate (12 strips) coated with a polyclonal antibody against human Apo A-1. 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-1 Standard: Human Apo A-1 in a buffered protein base (160 µg, lyophilized). Biotinylated Apo A-1: 1 vial, lyophilized. MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered

Product Details

	surfactant (30 ml). 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:	APOA1
Alternative Name:	Apolipoprotein A-I (APOA1 Products)
Background:	Human apolipoprotein A-I (Apo A-1) comprises about 70% of the high-density lipoproteins (HDL) protein mass and Apo A-1I another 15-20%. Apo A-1, a 243-amino acid molecule that contains a series of highly homologous amphipathic alpha-helices, is a 28-kDa single polypeptide that lacks glycosylation or disulfide linkages. About 5-10% of human plasma Apo A-1 exists in a lipoprotein-unassociated state. Apo A-1 appears to have effects on the atherosclerosis inhibition, reverse cholesterol transport and anti-inflammation. Oxidation of specific amino acid residues in Apo A-1 may contribute to atherogenesis by impairing cholesterol efflux from macrophages. A majority of HDL functionality is derived from the ability of Apo A-1 to sequester phospholipid and cholesterol and interact with plasma enzymes and cellular receptors. During reverse cholesterol transport, HDL interacts with lecithin:cholesteryl acyltransferase (LCAT) and cellular receptors, including ATP-binding cassette transporter protein A-1 (ABCA1) and the scavenger receptor class B type I in an ordered fashion that is reflected by HDL particle lipid composition. A high-affinity HDL receptor for Apo A-1 is beta-chain of ATP synthase on the surface of hepatocytes. The plasma concentration of Apo A-1 is one of the best indicators of susceptibility to cardiovascular disease.
Pathways:	Regulation of Lipid Metabolism by PPARalpha, Production of Molecular Mediator of Immune Response, Lipid Metabolism

Application Details

Sample Volume:	25 µL
Assay Time:	< 3 h
Plate:	Pre-coated

Application Details

Protocol:	This assay employs a quantitative competitive enzyme immunoassay technique that measures human Apo A-1 in less than 3 hours. A polyclonal antibody specific for human ApoA-1 has been pre-coated onto a 96-well microplate with removable strips. Apo A-1 in standards and samples is competed with a biotinylated Apo A-1 sandwiched by the immobilized antibody and 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. MIx Diluent Concentrate (10x): Dilute the MIx Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 160 g of Apo A-1 Standard with 4 ml of MIx Diluent to generate a solution of 40 g/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (40 g/ml) 1:2 with equal volume MIx Diluent to produce 20, 10, 5, 2.5 and 1.25 g/ml solutions. MIx Diluent serves as the zero standard (0 g/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Apo A-1] (g/ml) 1 part Standard (40 g/ml) + 1 part MIx Diluent P1 20.0 P2 1 part P1 + 1 part MIx Diluent 10.0 P3 1 part P2 + 1 part MIx Diluent 5.00 P4 1 part P3 + 1 part MIx Diluent 2.50 P5 1 part P4 + 1 part MIx Diluent 1.25 P6 MIx Diluent 0.00 Biotinylated Apo A-1 (4x): Dilute Biotinylated Apo A-1 with 4 ml MIx Diluent to produce a 4-fold stock solution. Allow to sit for 10 minutes with gentle agitation prior to use. The stock solution should be further diluted 1:4 with MIx 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 MIx 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 MIx Diluent or within the range 1:100 - 1:800. The user should determine the optimal dilution factor. 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 1:200 into MIx Diluent or within the range 1:100-1:800. The user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. 2

Application Details

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 25 µL of standard or sample per well and immediately add 25 µL of Biotinlylated ApoA-1 to each well (on top of the Standard or sample) and mix gently. Cover wells with a 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 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 µL of Chromogen Substrate per well and incubate for about 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 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 and draw a best-fit curve through the points on the graph. Plotting the log-log graph may linearize the data and the best-fit line can be determined by regression analysis of the linear portion of the curve. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. 3 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 4.6% and 7.3% respectively.
Restrictions:	For Research Use only

Handling

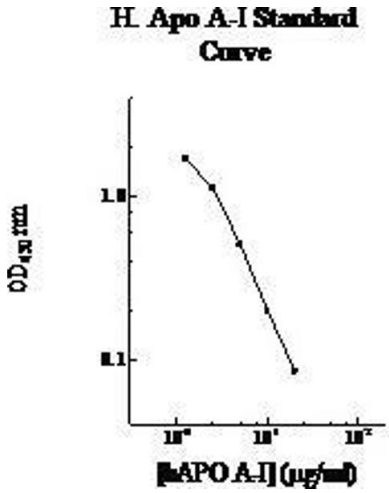
Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- protein, and

Handling

	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 protocol. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial before opening and using contents. 1 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. Store SP Conjugate at -20°C Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C. Store Standard and Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Publications

Product cited in:	Kootte, Smits, van der Valk, Dasseux, Keyserling, Barbaras, Paolini, Santos, van Dijk, Dallinga-van Thie, Nederveen, Mulder, Hovingh, Kastelein, Groen, Stroes: "Effect of open-label infusion of an apoA-I-containing particle (CER-001) on RCT and artery wall thickness in patients with FHA." in: **Journal of lipid research**, Vol. 56, Issue 3, pp. 703-12, (2015) (PubMed). Cubedo, Padro, Garcia-Arguinzonis, Vilahur, Minambres, Pou, Ybarra, Badimon: "A novel truncated form of Apolipoprotein A-I transported by dense LDL is increased in diabetic patients. " in: **Journal of lipid research**, (2015) (PubMed). Wu, Zhong, Hong, Li, Liu, Pan, Xin, Zhu: "The effects of Zanthoxylum bungeanum extract on lipid metabolism induced by sterols." in: **Journal of pharmacological sciences**, Vol. 127, Issue 3, pp. 251-9, (2015) (PubMed). Taylor, Plaisance, Mahurin, Mestek, Moncada-Jimenez, Grandjean: "Paraoxonase responses to exercise and niacin therapy in men with metabolic syndrome." in: **Redox report : communications in free radical research**, Vol. 20, Issue 1, pp. 42-8, (2014) (PubMed).



ELISA

Image 1.

