Datasheet for ABIN612662

APOA1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	APOA1
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	3 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Apo A-I ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo A-I in urine, saliva, milk, and cell culture supernatant
Brand:	AssayMax
Sample Type:	Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	No significant cross reactivity with Apo AII, Apo B, Apo CI, Apo CII, Apo CIII or Apo E.
Components:	Human Apo A-I Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo A-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. Human Apo A-I Standard: Human Apo A-I in a buffered protein base (3.2 µg, lyophilized). Biotinylated Apo A-I Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Apo A-I (80µl). MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml).

Product Details

	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:	APOA1
Alternative Name:	Apolipoprotein A-I (APOA1 Products)
Background:	Human apolipoprotein A-I (Apo A-I) comprises about 70% of the high-density lipoproteins (HDL) protein mass and Apo A-II another 15-20%. Apo A-I, 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-I exists in a lipoprotein-unassociated state. Apo A-I appears to have effects on the atherosclerosis inhibition, reverse cholesterol transport and anti-inflammation. Oxidation of specific amino acid residues in Apo A-I may contribute to atherogenesis by impairing cholesterol efflux from macrophages. A majority of HDL functionality is derived from the ability of Apo A-I 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-I is beta-chain of ATP synthase on the surface of hepatocytes. The plasma concentration of Apo A-I 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:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated

Application Details

Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Apo A-I in less than 4 hours. A polyclonal antibody specific for human Apo A-1 has been pre-coated onto a 96-well microplate with removable strips. Apo A-1 in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Apo A-1, 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. 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 3.2 g of Apo A-I Standard with 4 ml of MIx Diluent to generate a stock solution of 800 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Further dilute the stock solution 1:4 with MIx to generate a standard solution of 200 ng/ml. Prepare duplicate or triplicate standard points by serially diluting the standard solution (200 ng/ml) 1:2 with equal volume of MIx Diluent to produce 100, 50, 25, 12.5, 6.25 and 3.13 ng/ml solutions. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Apo A-I] (ng/ml) P1 1 part Stock (800ng/ml) + 3 parts MIx Diluent 200.00 P2 1 part P1 + 1 part MIx Diluent 100.00 P3 1 part P2 + 1 part MIx Diluent 50.00 P4 1 part P3 + 1 part MIx Diluent 25.00 P5 1 part P4 + 1 part MIx Diluent 12.50 P6 1 part P5 + 1 part MIx Diluent 6.25 P7 1 part P6 + 1 part MIx Diluent 3.13 P8 MIx Diluent 0.00 Biotin Apo A-I Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 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:	Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:4 into MIx Diluent. 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. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:8 into MIx Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. 2 Milk: Collect milk using sample tube. 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.

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 50 µL of Apo A-I standard or sample per well. 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 Biotinylated Apo A-I Antibody to each well and incubate for one hour. Wash the microplate as described above. 3 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 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 duplicate or triplicate readings for each standard and sample. To generate Standard Curve, plot 4-parameter graph or semi-log graph using the Angiotensin II 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 4.7% and 7.2% respectively.
Restrictions:	For Research Use only

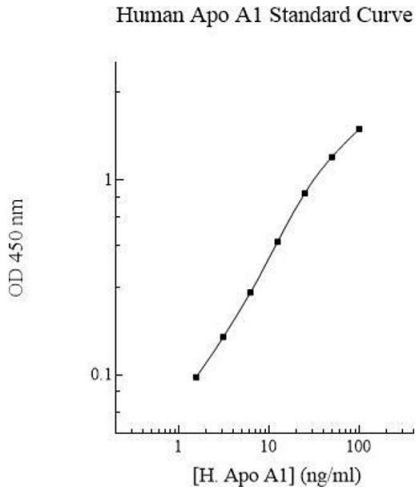
Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- antibody, 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 and the biotinylated-antibody 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 and Biotinylated Antibody 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 at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Validation report #102976 for ELISA (ELISA)



ELISA

Image 1.

Validation report #102975 for ELISA (ELISA)

Successfully validated (ELISA (ELISA))

by The Durham Genome Center

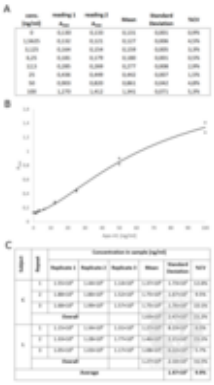
Report Number: 102975

Date: Mar 13 2018

Target:	APOA1
Lot Number:	3821705
Method validated:	ELISA (ELISA)
Positive Control:	The measurements of Apolipoprotein Apo A-I were performed directly on human serum samples.
Negative Control:	Standard curve negative control
Notes:	Passed. The human Apo A-I ELISA kit ABIN612662 specifically detects Apo A-I in serum samples.
Protocol:	• Prepare two human samples and freeze in aliquots. Carry out three repeat measurements of each sample using three replicates from two subjects. • Prepare all the reagents, standard solutions and samples as instructed by the manufacturer. • Prepare standards and samples in triplicates. • Add 50µl of standard or sample per well. • Incubate plate for 2h at RT. • Wash plate as instructed then add 50µl of Biotinylated Antibody per well. • Incubate plate for 1h at RT. • Wash plate as instructed then add 50µl of SP Conjugate per well. • Incubate plate for 30min at RT. • Wash plate as instructed, add 50µl of Chromogen Substrate per well. • Incubate plate for 20min at RT. • Add 50µl of Stop Solution per well and read absorbance at 450nm immediately.
Experimental Notes:	• The purpose of this experiment is to validate the measurement of Apo A-I in frozen Human Serum Samples. ABIN612662 measures Apo A-I in an assay range from 1.563ng/ml to 100ng/ml. For human serum a sample dilution of 1:100000 is recommended. However, we found that a 1:20000 dilution was more appropriate for our samples. The average human value is reported to be 1.1mg/ml (i.e. 1.1×10^6ng/ml). • The mean values for the two samples 1.63×10^6 and 1.27×10^6, are 48% and 16% higher than the manufacturer's reported average human serum value of 1.1×10^6ng/ml (as reported in the assay instruction manual), but are consistent with the reported range of values in clinical samples (Contois et al. (1996)).

- The standard curves for the assays reported were fitted with a 4-Parameter Logistic model, giving good quality fits (R^2>0.95) (Panel B). All statistical calculations were performed in Microsoft Excel 2016. ANOVA was performed using the Excel Data Analysis Toolpak.
- All absorbance values fell within the range of the standard curve (Panel A). The assay precision experiment yielded %CVs within the target range (<10%) (Panel C).

Image for Validation report #102975



Validation image no. 1 for Apolipoprotein A-I (APOA1) ELISA Kit (ABIN612662)

A. Measurement of A_{450} values for the standards provided with the kit. B. Standard curve and 4-Parameter Logistic Fit. For this example, R^2=0.9942. C. Assay Precision results. Three repeats with three replicates each were performed for two samples coded C and L.

