Datasheet for ABIN612664

APOB ELISA Kit

1 Validation | 1 Image | 3 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	APOB
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.008 µg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Apo B ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo B in plasma, serum and cell culture samples
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 AII, Apo CI, Apo CII, Apo CIII or Apo E.
Components:	Human Apo B Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo B. 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 B Standard: Human Apo B in a buffered protein base (0.75 ug, lyophilized). Biotinylated Apo B Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Apo B (80µl). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash

Product Details

	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:	APOB
Alternative Name:	Apolipoprotein B (APOB Products)
Background:	Apolipoprotein B (Apo B) is the dominant protein constituent of LDL. The levels of secreted Apo B directly correlate with circulating serum cholesterol levels. Apo B is a better marker of risk of vascular disease than other lipid markers including LDL and HDL-cholesterol and triglycerides. Apo B is consistently associated with an increased mortality in type 1 diabetes. Plasma apo B and VLDL and LDL with apo B are independent risk factors for cardiovascular disease (CVD). Apo B, Apo A-I and the Apo A-I/Apo B ratio can predict incident ischemic stroke among patients with preexisting atherothrombotic disease.
Pathways:	Lipid Metabolism

Application Details

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 B in less than 4 hours. A polyclonal antibody specific for human Apo B has been pre-coated onto a 96-well microplate with removable strips. Apo B in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Apo B, 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. EIA Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have

	completely dissolved. Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 0.75 ug of Apo B Standard with 3 ml of EIA Diluent to generate a solution of 0.25 ug/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 (0.25 ug/ml) 1:2 with EIA Diluent to produce 0.125, 0.063, 0.031, 0.016, and 0.008 ug/ml solutions. EIA Diluent serves as the zero standard (0 ug/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Apo B] (ug/ml) P1 1 part Standard Stock (0.25 g/ml) 0.250 P2 1 part P1 + 1 part EIA Diluent 0.125 P3 1 part P2 + 1 part EIA Diluent 0.063 P4 1 part P3 + 1 part EIA Diluent 0.031 P5 1 part P4 + 1 part EIA Diluent 0.016 P6 1 part P5 + 1 part EIA Diluent 0.008 P7 EIA Diluent 0.000 Biotinylated Apo B 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): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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 1:20000 into EIA Diluent. If necessary dilute samples within the range of 1:10000 to 1:40000. Store samples 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. Dilute samples 1:20000 into EIA Diluent. If necessary dilute samples within the range of 1:10000 to 1:40000. 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 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. 2
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 B 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

Application Details

	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 B Antibody to each well and incubate for one hour. Wash the 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. 3 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. The best-fit line can be determined by regression analysis using log-log or 4-parameter curve fit. 3 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 5.0% and 7.5% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- antibody, and 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. This kit is for research use only. The kit should not be used beyond the expiration date. The Stop Solution is an acidic solution.
Storage:	4 °C/-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.

Handling

Store Standard, SP Conjugate, and Biotinylated Antibody 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.

Publications

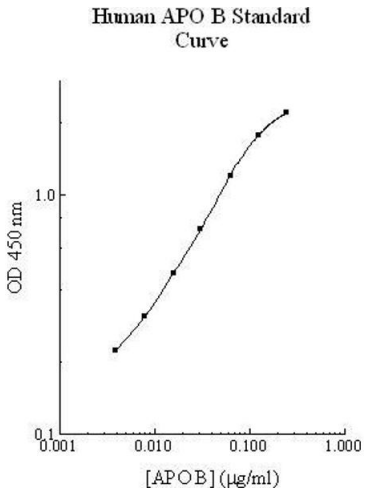
Product cited in:

Tomassen, Govers, Vos, de Wit: "Dietary fat induced chylomicron-mediated LPS translocation in a bicameral Caco-2cell model." in: **Lipids in health and disease**, Vol. 22, Issue 1, pp. 4, (2023) (PubMed).

Cha, Niedzwiecki, Rath: "Hypoascorbemia induces atherosclerosis and vascular deposition of lipoprotein(a) in transgenic mice." in: **American journal of cardiovascular disease**, Vol. 5, Issue 1, pp. 53-62, (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).

Images



ELISA

Image 1.

Successfully validated (ELISA (ELISA))

by The Durham Genome Center

Report Number: 102976

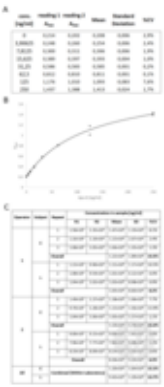
Date: Mar 13 2018

Target:	APOB
Lot Number:	1981806
Method validated:	ELISA (ELISA)
Positive Control:	The measurements of Apolipoprotein B-100 were performed directly on human serum samples.
Negative Control:	Standard curve negative control
Notes:	Passed. The human Apo B-100 ELISA kit ABIN612664 specifically detects Apo B-100 in serum samples.
Protocol:	• Prepare two human samples and freeze in aliquots. Carry out three repeat measurements of each sample using two 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 8min at RT. • Add 50µl of Stop Solution per well and read absorbance at 450nm immediately.
Experimental Notes:	• The purpose of this report is to validate the measurement of Apolipoprotein-B in frozen Human Serum Samples. ABIN612664 measures Apo B-100 within a assay range of 3.906ng/ml to 250ng/ml. The manufacturer reports an average concentration in human serum of 0.6–1.6mg/ml (0.6×10^6 to 1.6×10^6ng/ml). • The reference range is less than 130mg/dl (Grundy SM (2002)) which is equivalent to 1.3×10^6ng/ml. For human serum, a sample dilution of 1:20000 is recommended (or in the range 1:10000 to 1:40000). However, we found that a 1:10000 dilution was more appropriate for our samples. • 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 within-laboratory precision values obtained for the two samples were 15.3% and 13.7% (average 14.5%). The values obtained for the two samples, 1.20×10^6 and 9.43×10^5, are consistent with the reported range of human serum values and fall into the reference range. These values also fall between 25% and 75% of the concentration range of the standard curve.
- The mean, standard deviation, and coefficient of variance (CV) are calculated for each repeat; overall statistics are also calculated across all nine replicates from each sample (Panel C). Additionally, the within laboratory precision is calculated using the values from both operators.

Image for Validation report #102976



Validation image no. 1 for Apolipoprotein B (APOB) ELISA Kit (ABIN612664)

A. Measurement of A_{450} values for the standards provided with ABIN612664. B. Representative standard curve and 4-Parameter Logistic Fit. For this example, R^2=0.9938. C. Assay Precision results (corrected for 1:10000 dilution) in ng/ml. Three repeats with two replicates each were performed for two samples coded C and L by two operators.