Datasheet for ABIN612665

APOE ELISA Kit

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Overview

Quantity:	96 tests
Target:	APOE
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	30 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Apo E ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo E in plasma, serum, urine, and cell culture samples
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The kit recognizes Apo E2, Apo E3, and Apo E4 isoforms.
Components:	Human Apo E Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo E. 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 E Standard: Human Apo E in a buffered protein base (8 µg, lyophilized). 1 Biotinylated Apo E Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Apo E (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:	APOE
Alternative Name:	Apolipoprotein E (APOE Products)
Background:	Apolipoprotein E (apoE) is a 34 kDa polymorphic protein with 299 amino acids and occurs in all lipoprotein fractions in plasma. It is synthesized primarily by the liver and is a main apoprotein of the chylomicron. ApoE is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and cardiovascular diseases. ApoE is also critical in several other important biological processes, including Alzheimer's disease, cognitive function, immunoregulation, cell signaling, and infectious diseases. There are three common isoforms of the protein: apoE3 is normal, while apoE2 and apoE4 are dysfunctional. ApoE deficiency causes type III hyperlipoproteinemia and premature atherosclerosis. ApoE is a major genetic risk factor for late-onset familial Alzheimer's disease and for cognitive deficits associated with aging (4-7). ApoE4 enhances HIV-1 cell entry in vitro and the ApoE epsilon4/epsilon4 genotype accelerates HIV disease progression.
Pathways:	Regulation of Cell Size, 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 Apo E in less than 4 hours. A polyclonal antibody specific for human Apo E has been pre-coated onto a 96-well microplate with removable strips. Apo E in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Apo E, 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 8 g of Apo E Standard with 4 ml of EIA Diluent to generate a solution of 2 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 (2 g/ml) 1:2 with EIA Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625 and 0.0313 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 E] (g/ml) Standard (2 g/ml) P1 2.000 P2 1 part P1 + 1 part EIA Diluent 1.000 P3 1 part P2 + 1 part EIA Diluent 0.500 P4 1 part P3 + 1 part EIA Diluent 0.250 P5 1 part P4 + 1 part EIA Diluent 0.125 P6 1 part P5 + 1 part EIA Diluent 0.063 P7 1 part P6 + 1 part EIA Diluent 0.031 P8 EIA Diluent 0.000 Biotin Apo E 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 1:400 into EIA Diluent as follows: add 10 µl of sample to 390 µl of EIA Diluent (1:40) to make Solution A, then add 80 µl of Solution A to 720 µl of EIA Diluent (1:10) to make a final working solution (1:400). 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 1:400 into EIA Diluent as follows: add 10 µl of sample to 390 µl of EIA Diluent (1:40) to make Solution A, then add 80 µl of Solution A to 720 µl of EIA Diluent (1:10) to make a final working solution (1:400). 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. Urine: Collect urine 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. 2
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room

Application Details

	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 E 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 E Antibody to each well and incubate for one hour. 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. 3 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 duplicate or 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 4.6% and 7.6% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C

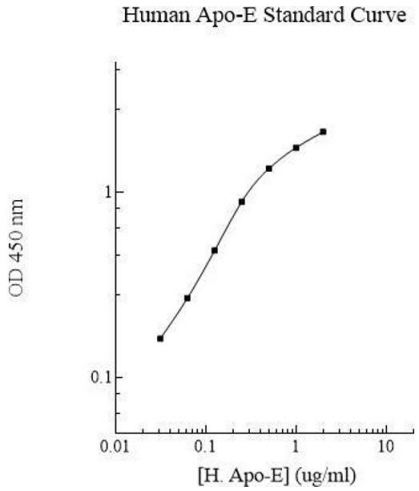
Handling

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.

Publications

Product cited in:	Felix, Hauck, Fritz, Hinz, Schnölzer, Kempf, Warnken, Michel, Pawlita, Werner: "Serum protein signatures differentiating autoimmune pancreatitis versus pancreatic cancer." in: **PLoS ONE**, Vol. 8, Issue 12, pp. e82755, (2013) (PubMed).

Images



ELISA

Image 1.

Successfully validated (ELISA (ELISA))

by The Durham Genome Center

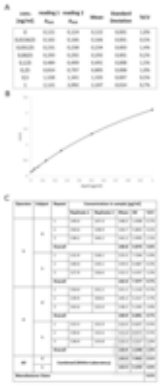
Report Number: 102977

Date: Mar 13 2018

Target:	APOE
Lot Number:	11021814
Method validated:	ELISA (ELISA)
Positive Control:	The measurements of Apolipoprotein E were performed directly on human serum samples.
Negative Control:	Standard curve negative control
Notes:	Passed. The human Apo-E ELISA kit ABIN612665 specifically detects Apo-E in human serum samples.
Protocol:	• Two human samples were prepared and frozen in aliquots. Two operators made three repeat measurements of the samples 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 15min 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 experiment is to validate the measurement of Apolipoprotein-E in frozen Human Serum Samples. ABIN612665 measures Apo-E within a range of 0.016µg/ml to 1.0µg/ml. For human serum, a sample dilution of 1:400 is recommended. This corresponds to a sample concentration range of 6.4µg/ml to 400µg/ml. The manufacturer reports an average concentration in human serum of 65µg/ml (n=10). The values measured for the samples are within the normal range of human values. • The standard curves for the assays reported were fitted with a 4-Parameter Logistic model, giving good quality fits (R^2=0.9997) (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 Apo-E assay

precision (represented by %CV values) was within the manufacturer's claimed range (Panel C). All absorbance values fell within the range of the standard curve. The within-laboratory precision obtained for each of the two samples was less than the manufacturer's claimed 4.5%.

Image for Validation report #102977



Validation image no. 1 for Apolipoprotein E (APOE) ELISA Kit (ABIN612665)

A. Measurement of A_{450} values for the standards provided with ABIN612665. B. Representative standard curve and 4-Parameter Logistic Fit. For this example, R^2=0.9997. C. Assay Precision results in µg/ml. Three repeats with two replicates each were performed for two samples coded C and L by two operators.