

Datasheet for ABIN1440225

APOA2 ELISA Kit[Go to Product page](#)**1** Image

Overview

Quantity:	96 tests
Target:	APOA2
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	~ 1.8 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 ApoA-II in plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human ApoA-II in less than 4 hours. A polyclonal antibody specific for human ApoA-II has been pre-coated onto a 96-well microplate with removable strips. ApoA-II in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for human ApoA-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.

Brand:	AssayMax
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric

Product Details

Cross-Reactivity (Details):	Cross-Reactivity: Monkey 10%. No significant cross reactivity observed with Apo AI, Apo B, Apo CI, Apo CII, Apo CIII, Apo E, Apo H, and Apo M.
Sensitivity:	High Sensitivity
Characteristics:	Standard Added Value: 3 - 30 ng/mL
Components:	Human Apo A-II Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo A-II. 2 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 ApoA-II in a buffered protein base (180 ng, lyophilized). Biotinylated Apo A-II Antibody (70x): A 70-fold concentrated biotinylated polyclonal antibody against Apo A-II (105 µL). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 mL). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 mL, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 µL). 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, 200-1000µL and multiple channel) Deionized or distilled reagent grade water

Target Details

Target:	APOA2
Alternative Name:	Apolipoprotein AII (APOA2 Products)
Background:	Apolipoprotein A-II (ApoA-II) is the second most abundant apolipoprotein 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. ApoA-II is found in plasma as a monomer, homodimer of 17.4 kDa, or heterodimer with ApoE and ApoD. 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. 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

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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](#)

Application Details

Assay Time: < 4 h

Plate: Pre-coated

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 180 ng of Apo A-II Standard with 1.5 mL of EIA Diluent to generate a working solution of 120 ng/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 (120 ng/mL) 1:2 with equal volume of EIA Diluent to produce 60, 30, 15, 7.5, 3.75, and 1.875 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within 30 days.

Biotinylated Apo A-II Antibody (70x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 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 Preparation: 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 1:20000 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 1:20000 into EIA Diluent. Store serum at -20°C or below. Avoid repeated freeze-thaw cycles

Cell Culture Media: 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.

Cell Lysate: Rinse cell with cold PBS and then Scrape the cell into a tube with 5 mL cold PBS with 0.5 M EDTA. Centrifuge suspension at 1,500 rpm for 10 minutes at 4°C and aspirate supernatant. Re-suspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH 8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1×10^6 cells add approximately 100 μ L of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13,000 rpm for 30 at 4°C and collect supernatant for assay.

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 μ L of Apo A-II 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 μ L 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 μ L 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 μ L of Biotinylated Apo A-II Antibody to each well and incubate for one hour.

Wash the microplate as described above.

Add 50 μ L 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 μ L of Chromogen Substrate per well and incubate for about 15 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 μ L 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

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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.

Assay Precision: Intra-assay and inter-assay coefficients of variation were 4.7% and 7.3% 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.

The kit should not be used beyond the expiration date.

The Stop Solution is an acidic solution

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

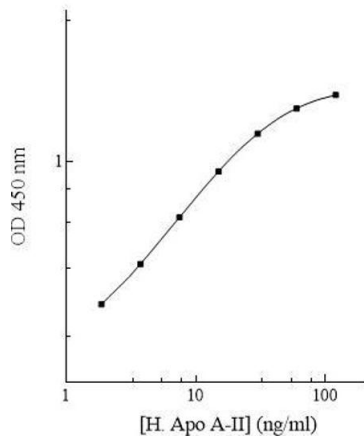
Opened unused microplate wells may be returned to the foil pouch with the desiccant packs.

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.

Human Apo A-II Standard Curve



ELISA

Image 1.