



AssayMax Human Apolipoprotein A-II ELISA Kit

Catalog No. EA5222-1

Introduction

Apolipoprotein A-II (ApoA-II) is the second most abundant apolipoproteins 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 (1 - 3). ApoA-II is found in plasma as a monomer, homodimer of 17.4 kDa, or heterodimer with ApoE and ApoD (4 - 7). 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 (7 - 12). ApoA-II is inversely associated with risk of future coronary artery disease (13). Serum levels of an isoform of ApoA-II have been identified as a potential marker for prostate cancer (14).

Principal of the Assay

The AssayMax Human Apo A-II ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo A-II in plasma, serum, urine, milk, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Apo A-II in less than 4 hours. A monoclonal antibody specific for human Apo A-II has been pre-coated onto a 96-well microplate with removable strips. Apo A-II in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for human Apo A-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.

Caution and Warning

- **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

Reagents

- **Human Apo A-II Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human Apo A-II.
- **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 Apo A-II in a buffered protein base (8 µg, lyophilized).
- **Biotinylated Apo A-II Antibody (25x):** A 25-fold concentrated biotinylated polyclonal antibody against Apo A-II (280 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).

Storage Condition

- Store components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20⁰C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8⁰C
- 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 2-8⁰C.
- Store Standard at -20⁰C before reconstituting with Diluent. Store Standard at -20⁰C for up to 1 month after reconstituting with Diluent.

Other Supplies Required

- 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

Sample Collection, Preparation and Storage

- **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 1:1000 with EIA Diluent and assay. The undiluted samples can be stored at -20⁰C 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 1:1000 into EIA Diluent. Store serum at -20⁰C or below. Avoid repeated freeze-thaw cycles
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples at -20⁰C or below for up to 3 months. 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 -20⁰C 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 min at 4⁰C and aspirate

supernatant. Re-suspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH8.0, 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 min. Centrifuge at 13,000 rpm for 30 at 4°C and collect supernatant for assay.

- **Milk:** Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes. Milk dilution is suggested at 1:2 in EIA Diluent; however, the user should determine the optimal dilution factor. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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 8 μ g of Apo A-II Standard with 1 ml of EIA Diluent to generate a standard stock of 8 μ g/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Dilute standard (8 μ g/ml) 1:2 with equal volume of EIA Diluent to generate a standard solution of 4 μ g/ml. Prepare duplicate or triplicate standard points by serially diluting the standard solution (4 μ g/ml) 1:4 with EIA Diluent to produce 1, 0.25, 0.063, 0.016 and 0.004 μ g/ml solutions. EIA Diluent serves as the zero standard (0 μ g/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[Apo A-II] (μ g/ml)
P1	Standard Stock (8 μ g/ml) + 1 part EIA Diluent	4.000
P2	1 part P1 + 3 parts EIA Diluent	1.000
P3	1 part P2 + 3 parts EIA Diluent	0.250
P4	1 part P3 + 3 parts EIA Diluent	0.063
P5	1 part P4 + 3 parts EIA Diluent	0.016
P6	1 part P5 + 3 parts EIA Diluent	0.004
P7	EIA Diluent	0.000

- **Biotinylated Apo A-II Antibody (25x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:25 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.

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.

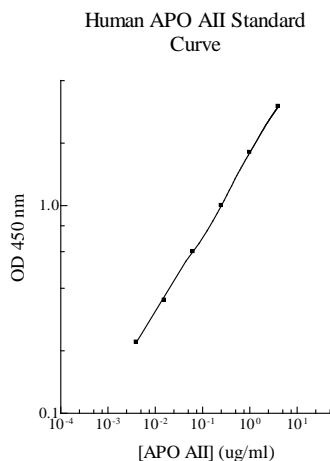
- Wash five times with 200 μ l of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
If using a machine wash six times with 300 μ l of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 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 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 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.

Data Analysis

- 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.



Performance Characteristics

- The minimum detectable dose of Apo A-II is typically \sim 0.004 μ g/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.2% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:500	83%	86%
1:1000	97%	99%
1:2000	110%	112%

Sample Dilution	Average Percentage of Expected Value	
	Urine	Milk
No Dilution	87%	92%
1:2	93%	98%
1:4	106%	101%

Recovery

Standard Added Value	0.02 – 2 µg/ml
Recovery %	82-106 %
Average Recovery %	97 %

Cross-Reactivity

Species	% Cross Reactivity
Canine	0.5 %
Bovine	None
Monkey	5%
Mouse	0.5%
Rat	0.1%
Swine	0.1%
Rabbit	None

- No significant cross reactivity with Apo AI, Apo B, Apo CI, Apo CII, Apo CIII or Apo E.

References

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