AssayMax Human Apolipoprotein A-I ELISA Kit (Plasma, Serum, and Cell Culture Supernatants)

Catalog Number EA5201-1

Introduction

Human apolipoprotein A-I (Apo A-1) comprises about 70% of the high-density lipoproteins (HDL) protein mass and Apo A-1I another 15–20%. Apo A-1, 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 (1). About 5–10% of human plasma Apo A-1 exists in a lipoprotein-unassociated state. Apo A-1 appears to have effects on the atherosclerosis inhibition, reverse cholesterol transport and anti-inflammation (2). Oxidation of specific amino acid residues in Apo A-1 may contribute to atherogenesis by impairing cholesterol efflux from macrophages (3). A majority of HDL functionality is derived from the ability of Apo A-1 to sequester phospholipid and cholesterol and interact with plasma enzymes and cellular receptors (4). 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-1 is beta-chain of ATP synthase on the surface of hepatocytes (5). The plasma concentration of Apo A-1 is one of the best indicators of susceptibility to cardiovascular disease (6).

Principal of the Assay

The AssayMax Human Apo A-1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo A-1 in plasma, serum and cell culture supernatants. This assay employs a quantitative competitive enzyme immunoassay technique that measures human Apo A-1 in less than 3 hours. A polyclonal antibody specific for human ApoA-1 has been pre-coated onto a 96-well microplate with removable strips. Apo A-1 in standards and samples is competed by a biotinylated Apo A-1 sandwiched by the immobilized antibody and 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

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution

Reagents

- **Human Apo A-1 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo A-1.
- **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-1 Standard:** Human Apo A-1 in a buffered protein base (80 µg, lyophilized).
- **Biotinylated Apo A-1:** 1 vial, lyophilized.
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 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 unopened kit at 2-8^{0}C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}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.

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 assay. Dilute samples 1:800 into MIX Diluent. The undiluted samples can be stored at -20^{0}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 1:800 into MIX Diluent. The undiluted samples can be stored at -20^{0}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^{0}C or below. Avoid repeated freeze-thaw cycles.

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^{0}C.

- **Standard Curve:** Reconstitute the 80 µg of Apo A-1 Standard with 4 ml of MIX Diluent to generate a solution of 20 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (20 µg/ml) 1:2 with equal volume MIX Diluent to produce 10, 5, 2.5, 1.25 and 0.625 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Apo A-1] (µg/ml)
P1	Standard (20 µg/ml)	20.000
P2	1 part P1 + 1 parts MIX Diluent	10.000
P3	1 part P2 + 1 parts MIX Diluent	5.000
P4	1 part P3 + 1 parts MIX Diluent	2.500
P5	1 part P4 + 1 parts MIX Diluent	1.250
P6	1 part P5 + 1 parts MIX Diluent	0.625
P7	MIX Diluent	0.000

- **Biotinylated Apo A-1 (2x):** Dilute Biotinylated Apo A-1 with 4 ml MIX Diluent to produce 2-fold stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution can be further diluted 1:2 with MIX Diluent. Any remaining solution should be frozen at -20^{0}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^{0}C.

Assay Procedure

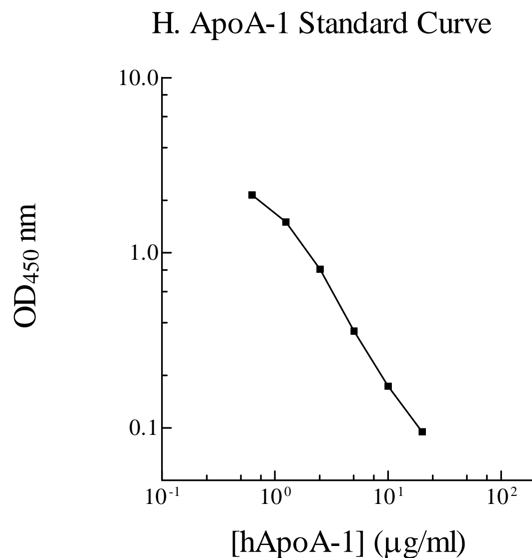
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of standard or sample per well, and immediately add 25 µl of Biotinlylated Apo A-1 to each well (on top of the Standard or sample) and mix gently. 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. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- 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 five times with 200 µl of Wash Buffer.
- 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**. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.

Data Analysis

- 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 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-1 is typically 100 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.3% respectively.
- No significant cross reactivity with Apo AII, Apo B, Apo CI, Apo CII, Apo CIII or Apo E.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:400	102%	103%
1:800	95%	100%
1:1600	96%	94%

Recovery

Standard Added Value	1 – 10 ug/ml
Recovery %	83-105 %
Average Recovery %	94 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 2
Bovine	None
Monkey	< 10 (suggest dilution 1:20 for plasma)
Mouse	None
Rat	None
Swine	< 2

Reference Value

- The normal blood levels of Apo A-1 is ranged from 90 – 130 mg/dL.

References

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(3) Shao B et al. (2006) Curr Opin Mol Ther. 8(3): 198-205.
(4) Forte T et al. (1971) Biochim. Biophys. Acta 248:381-386.
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(6) Noma A et al. (1983) Atherosclerosis 49:1-7.

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