Human Apolipoprotein A-I ELISA Kit
(Urine, Saliva, Milk and Cell Culture Supernatants)

Introduction

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

Principal of the Assay

The Human Apo A-I ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Apo A-I in urine, saliva, milk, and cell culture supernatant. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Apo A-I in less than 4 hours. A polyclonal antibody specific for human Apo A-1 has been pre-coated onto a 96-well microplate with removable strips. Apo A-1 in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Apo A-1, 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

- 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-I Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo A-I.
- **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-I Standard:** Human Apo A-I in a buffered protein base (400 ng, lyophilized).
- **Biotinylated Apo A-I Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against Apo A-I (80 µl).
- **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, 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 kit at 2-8^{0}C or -20^{0}C upon arrival up to the 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

- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:4 into MIX Diluent. Store samples 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.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:8 into MIX Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples at -20^{0}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. 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 400 ng of Apo A-I Standard with 2 ml of MIX Diluent to generate a solution of 200 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 (200 ng/ml) 1:2 with equal volume of MIX Diluent to produce 100, 50, 25, 12.5, 6.25 and 3.13 ng/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-I] (ng/ml)
P1	Standard (200 ng/ml)	200.000
P2	1 part P1 + 1 part MIX Diluent	100.000
P3	1 part P2 + 1 part MIX Diluent	50.000
P4	1 part P3 + 1 part MIX Diluent	25.000
P5	1 part P4 + 1 part MIX Diluent	12.500
P6	1 part P5 + 1 part MIX Diluent	6.250
P7	1 part P6 + 1 part MIX Diluent	3.130
P8	MIX Diluent	0.000

- **Biotin Apo A-I Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 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 50 µl of Apo A-I 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 A-I 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.

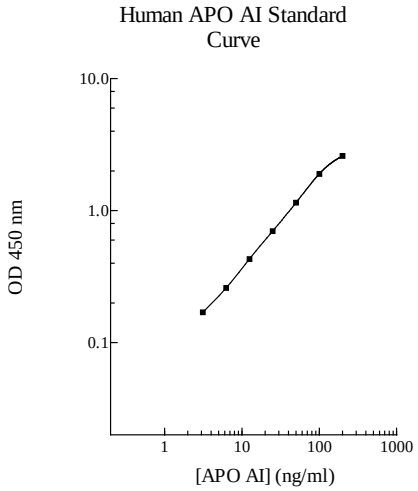
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. 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.

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-I is typically less than 3 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	**Urine**	**Saliva**
1:2	105%	
1:4	100%	94%
1:8	94%	98%
1:16		97%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
No Dilution	97%
1:2	94%
1:4	95%

Recovery

Standard Added Value	10 – 100 ng/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

References

(1) Davidson WS and Thompson TB (2007) *J. Biol. Chem.* 282 (31): 22249-22253.
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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.
(5) Martinez LO *et al.* (2003) *Nature* 421(6918): 75-79.
(6) Noma A *et al*. (1983) *Atherosclerosis* 49:1-7.