Human ApoA-I
ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

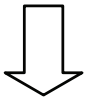
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

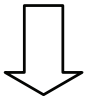
Thank you for choosing Assaypro.

Assay Summary

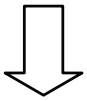
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



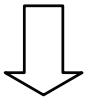
Wash, then add 50 µl of
Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add 50 µl of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 20 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

AssayMax Human Apolipoprotein A-I ELISA Kit

Catalog No. EA5301-1
Sample Insert/Reference Only

Introduction

Human Apolipoprotein A-I (ApoA-I) comprises about 70% of the high-density lipoprotein's (HDL) protein mass, while ApoA-II comprises 15 – 20%. ApoA-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 ApoA-I exists in a lipoprotein unassociated state. ApoA-I appears to have effects on the atherosclerosis inhibition, reverse cholesterol transport, and anti-inflammation (2). Oxidation of specific amino acid residues in ApoA-I may contribute to atherogenesis by impairing cholesterol efflux from macrophages (3). A majority of HDL functionality is derived from the ability of ApoA-I to sequester phospholipids and cholesterol as well as 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-I (ABCA1) and the scavenger receptor class B type I, in an ordered fashion that is reflected by HDL particle lipid composition. The beta-chain of ATP synathase, found on the surface of hepatocytes, contains a high-affinity HDL receptor for ApoA-1 (5). The plasma concentration of ApoA-I is one of the best indicators of susceptibility to cardiovascular disease (6).

Principle of the Assay

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

- **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 ApoA-I Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human ApoA-I.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human ApoA-I Standard:** Human ApoA-I in a buffered protein base (3.2 µg, lyophilized).
- **Biotinylated Human ApoA-I Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against ApoA-I (140 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

- Upon arrival, immediately store components of the kit at recommended temperatures 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.

- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, 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 and assay. The undiluted samples can be stored at -20°C 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 -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 1500 rpm for 10 minutes at 4°C 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 x 10^6 cells, add approximately 100 µL of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant for assay.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:8 into MIX Diluent and assay. The undiluted samples can be stored at -20°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, dilute if necessary and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:40 into MIX Diluent and assay. The undiluted samples can be stored at -80°C 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.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 3.2 µg of Human ApoA-I Standard with 4 ml of MIX Diluent to generate an 800 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Further dilute the standard stock solution 1:4 with MIX Diluent to generate a 200 ng/ml standard working solution. Prepare duplicate or triplicate standard points by serially diluting the standard working solution (200 ng/ml) 1:2 with equal volume of MIX Diluent to produce 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[ApoA-I] (ng/ml)
P1	1 part Standard (800 ng/ml) + 3 parts MIX Diluent	200.0
P2	1 part P1 + 1 part MIX Diluent	100.0
P3	1 part P2 + 1 part MIX Diluent	50.00
P4	1 part P3 + 1 part MIX Diluent	25.00
P5	1 part P4 + 1 part MIX Diluent	12.50
P6	1 part P5 + 1 part MIX Diluent	6.250
P7	1 part P6 + 1 part MIX Diluent	3.125
P8	MIX Diluent	0.000

- **Biotinylated Human ApoA-I Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX 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 MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μl of Human ApoA-I Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μl of Biotinylated Human ApoA-I Antibody to each well and incubate for 1 hour.
- Wash the 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 the 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.

Data Analysis

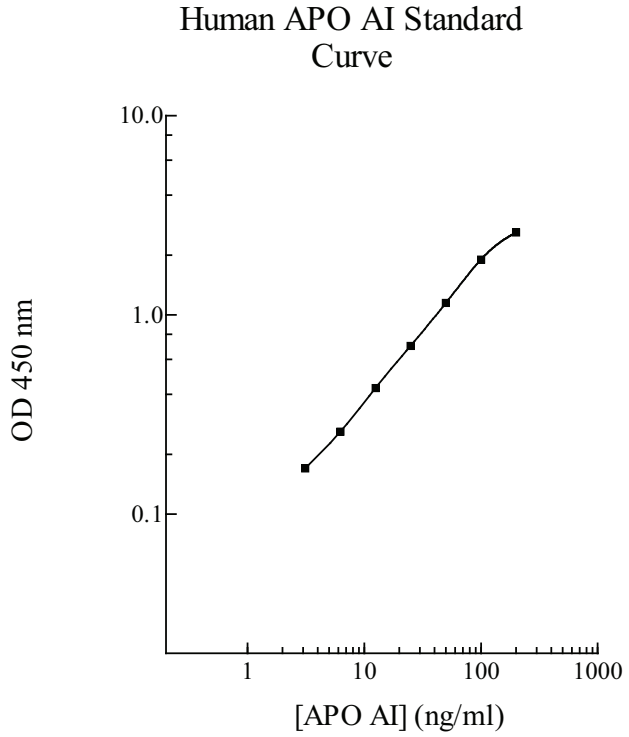
- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm

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 ApoA-I is typically ~ 3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.2% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Urine**	**Saliva**
1:2	98%	-
1:4	98%	96%
1:8	105%	99%
1:16	-	104%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
No Dilution	104%
1:2	98%
1:4	96%

Recovery

Standard Added Value	6.25 – 100 ng/ml
Recovery %	82 – 109%
Average Recovery %	97%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	<10%
Mouse	None
Rat	None
Swine	None
Rabbit	None

- No significant cross reactivity observed with ApoA-II, ApoB, ApoC-I, ApoC-II, ApoC-III, ApoH, and ApoE.

References

(1) Davidson WS and Thompson TB (2007) *J. Biol. Chem*. 282 (31): 22249-22253.
(2) Nessen SE *et al.* (2003) *J. Am. Med. Assoc.* 290**:** 2292–2300.
(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.

Version 3.0

Related products

- EA5201-1 AssayMax Human Apolipoprotein A-I (Plasma and Serum samples)