



# Human Apolipoprotein A-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.

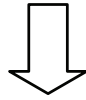
For any questions regarding troubleshooting or performing the assay, please contact our support team at [support@assaypro.com](mailto:support@assaypro.com).

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## Assay Summary

Add 25  $\mu$ l of Standard/ Sample and  
25  $\mu$ l of Biotinylated Protein per well.

Incubate 2 hours.



Wash, then add 50  $\mu$ l of  
SP Conjugate per well.

Incubate 30 minutes.



Wash, then add 50  $\mu$ l of  
Chromogen Substrate per well.

Incubate 15 minutes.



Add 50  $\mu$ l of Stop Solution per well.  
Read at 450 nm immediately.





# **AssayMax Human Apolipoprotein A-I ELISA Kit**

Catalog No. EA5201-1  
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 synthase, 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 plasma and serum samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures human apoA-I in less than 3 hours. A polyclonal antibody specific for human apoA-I has been pre-coated onto a 96-well microplate with removable strips. ApoA-I in standards and samples is competed with a biotinylated apoA-I 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

- Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated protein, 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 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) 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 (80 µg, lyophilized).
- **Biotinylated ApoA-I Protein:** 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 (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 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 and Biotinylated Protein 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  $\mu$ l, 20-200  $\mu$ l, 200-1000  $\mu$ l, 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 3000 x *g* for 10 minutes. Dilute samples 1:200 into MIX Diluent or within the range of 1:100 - 1:800, and assay. User should determine the optimal dilution factor. The undiluted samples can be stored at -20°C and below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute samples 1:200 into MIX Diluent or within the range of 1:100 - 1:800, and assay. User should determine the optimal dilution factor. The undiluted samples can be stored at -20°C and 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.
- **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 80  $\mu$ g of Human ApoA-I Standard with 4 ml of MIX Diluent to generate a 20  $\mu$ g/ml standard solution. 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 (20  $\mu$ g/ml) 1:2 with equal volume MIX Diluent to produce 10, 5, 2.5, and 1.25  $\mu$ g/ml solutions. MIX Diluent serves as the zero standard (0  $\mu$ g/ml). Any remaining solution should be frozen at -20°C and used within 10 days.

Standard Point	Dilution	[ApoA-I] ( $\mu\text{g/ml}$ )
P1	Standard (20 $\mu\text{g/ml}$ )	20.0
P2	1 part P1 + 1 part MIX Diluent	10.0
P3	1 part P2 + 1 part MIX Diluent	5.00
P4	1 part P3 + 1 part MIX Diluent	2.50
P5	1 part P4 + 1 part MIX Diluent	1.25
P6	MIX Diluent	0.00

- **Biotinylated Human ApoA-I Protein (2x):** Reconstitute Biotinylated ApoA-I Protein with 4 ml MIX Diluent to produce a 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2 with MIX Diluent. Any remaining solution should be frozen at  $-20^{\circ}\text{C}$  and used within 10 days.
- **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  $-20^{\circ}\text{C}$ .

## 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 ( $20-25^{\circ}\text{C}$ ).
- 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 25  $\mu\text{l}$  of Human ApoA-I Standard or sample per well and immediately add 25  $\mu\text{l}$  of Biotinylated Human ApoA-I Protein to each well (on top of the standard or sample) and mix gently. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu$ 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  $\mu$ 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.

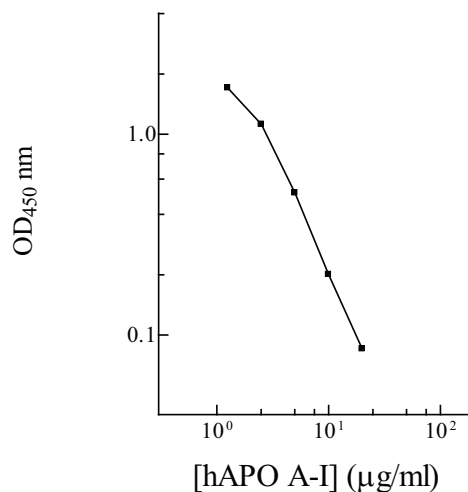
## Data Analysis

- 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 four-parameter or log-log 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.

H. Apo A-I Standard Curve



## Performance Characteristics

- The minimum detectable dose of apoA-I is typically ~ 1 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.2% respectively.

## Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:100	92%	91%
1:200	98%	100%
1:400	104%	106%

## Recovery

Standard Added Value	2 – 10 µg/ml
Recovery %	84 – 113%
Average Recovery %	98%

## Reference Value

- The normal blood levels of apoA-I is ranged from 90 – 130 mg/dL.

## Cross-Reactivity

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

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

## 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 3.5

## Related products

- EA5301-1 AssayMax Human Apolipoprotein A-I ELISA Kit (Urine, Milk, Saliva, and Cell Culture samples)