



Human Lp(a) 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 μ l of Standard/ Sample per well.
Incubate 2 hours.



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



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



Wash, then add 50 μ l of
Chromogen Substrate per well.
Incubate 10 minutes.



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

AssayMax Human Lipoprotein(a) ELISA Kit

Catalog No. EL3001-1
Sample Insert/Reference Only

Introduction

Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein particle formed by an assembly of LDL particles and apo(a) bound to apoB-100 component of LDL (1). Apo(a), the main constituent of Lp(a), has serine proteinase activity and is capable of autoproteolysis (2). Apo(a) has 4548 amino acids, variable sizes from 200 to 700 kDa, multiple isoforms, and structural homology with plasminogen (3-5). It competes with plasminogen for its binding site, inhibiting tissue-type plasminogen activator 1 and leading to reduced fibrinolysis (6). The mean Lp(a) protein level ranged from 78 to 175 $\mu\text{g/ml}$ depending on populations and the risk threshold is 300 $\mu\text{g/ml}$ (7-9). High levels of Lp(a) in the blood is a risk factor for myocardial infarction (MI), coronary heart disease (CHD), cerebrovascular disease (CVD), atherosclerosis, thrombosis, and stroke (10, 11).

Principle of the Assay

The AssayMax Human Lp(a) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Lp(a) in plasma, serum, urine, milk, CSF, and cell culture supernatant samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Lp(a) in less than 4 hours. A polyclonal antibody specific for human Lp(a) has been pre-coated onto a 96-well microplate with removable strips. Lp(a) in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Lp(a), 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 Lp(a) Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Lp(a).
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Lp(a) Standard:** Human Lp(a) in a buffered protein base (50 ng, lyophilized).
- **Biotinylated Human Lp(a) Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against Lp(a) (140 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

- 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 μ l, 20-200 μ l, 200-1000 μ 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 1:8000 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or 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. Dilute samples 1:8000 into EIA 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 Supernatants:** 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.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:4 into EIA 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 samples 1:4000 into EIA Diluent and assay. The undiluted 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 tube. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:8 into EIA 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.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.

- **Standard Curve:** Reconstitute the 50 ng of Human Lp(a) Standard with 1 ml of EIA Diluent to generate a 50 ng/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 (50 ng/ml) 1:2 with equal volume of EIA Diluent to produce 25, 12.5, 6.25, 3.125, 1.563, and 0.781 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 15 days.

Standard Point	Dilution	[Lp(a)] (ng/ml)
P1	Standard (50 ng/ml)	50.00
P2	1 part P1 + 1 part EIA Diluent	25.00
P3	1 part P2 + 1 part EIA Diluent	12.50
P4	1 part P3 + 1 part EIA Diluent	6.250
P5	1 part P4 + 1 part EIA Diluent	3.125
P6	1 part P5 + 1 part EIA Diluent	1.563
P7	1 part P6 + 1 part EIA Diluent	0.781
P8	EIA Diluent	0.000

- **Biotinylated Human Lp(a) Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 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, 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 Lp(a) 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 μ 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 μ l of Biotinylated Human Lp(a) Antibody to each well and incubate for 1 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.

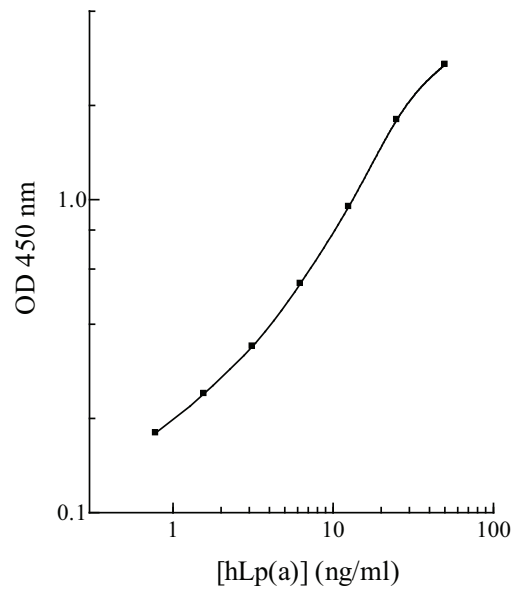
Data Analysis

- 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.

Human Lp(a) Standard Curve



Performance Characteristics

- The minimum detectable dose of Lp(a) is typically ~ 0.7 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.0% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value		
	Plasma	Serum	Milk
1:4000	93%	92%	98%
1:8000	100%	99%	101%
1:16000	107%	106%	106%

Sample Dilution	Average Percentage of Expected Value
	Urine
1:2	89%
1:4	97%
1:8	105%

Recovery

Standard Added Value	1.56 – 25 ng/ml
Recovery %	84 – 111%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	<10%
Mouse	None
Rat	None
Swine	None
Rabbit	None
Human	100%
Proteins	% Cross Reactivity
Apo B	10%

- No significant cross reactivity with human ApoA-I, ApoA-II, ApoC-I, ApoC-III, or ApoE.

Reference Values

- Normal human Lp(a) plasma levels range from 60 – 180 µg/ml.

References

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Version 2.2