Innovative RESEARCH Results Driven Research

Human Lipoprotein(a) [Lp(a)] ELISA Kit

Introduction

Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein particle formed by 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 able 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 µg/ml depending on populations and the risk threshold is 300 µg/ml (7 - 9). High level of Lp(a) in blood is a risk factor for myocardial infarction (MI), coronary heart disease (CHD), cerebrovascular disease (CVD), atherosclerosis, thrombosis, and stroke (10 - 11).

Principal of the Assay

The Human Lp(a) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Lp(a) in plasma, serum, urine, milk, and cell culture 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 acid 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 pre-cut, 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 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

- Store components of the kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20^{0}C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8^{0}C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8^{0}C.
- Store Standard at 2-8^{0}C before reconstituting with Diluent and at -20^{0}C after reconstituting with Diluent. Any remaining solution should be frozen at -20^{0}C and used within 5 days.

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 1:8000 into EIA Diluent as follows: add 5 µl of sample to 395 µl of EIA Diluent (1:80) to make Solution A; then add 8 µl of Solution A to 792 µl of EIA Diluent (1:100) to make a final working solution (1:8000). Store samples 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. Dilute samples 1:8000 into EIA Diluent as follows: add 5 µl of sample to 395 µl of EIA Diluent (1:80) to make Solution A; then add 8 µl of Solution A to 792 µl of EIA Diluent (1:100) to make a final working solution (1:8000). 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 2000 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.

- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes. Dilute samples 1:4 into EIA Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Centrifuge samples at 600 x g for 10 minutes. Dilute samples 1:4000 into EIA Diluent. 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.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 50 ng of Lp(a) Standard with 1 ml of EIA Diluent to generate a solution of 50 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 (50 ng/ml) 1:2 with equal volume of EIA Diluent to produce 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C and used within 5 days.

Standard Point	Dilution	[Lp(a)] (ng/ml)
P1	Standard solution (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	6.25
P5	1 part P4 + 1 part EIA Diluent	3.13
P6	1 part P5 + 1 part EIA Diluent	1.56
P7	1 part P6 + 1 part EIA Diluent	0.78
P8	EIA Diluent	0.00

- **Biotin 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^{0}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^{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 Lp(a) 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 Lp(a) Antibody to each well and incubate for one hour.

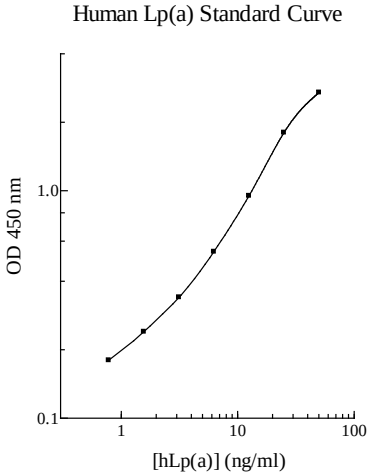
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 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**. 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 Lp(a) is typically ~ 0.8 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.6% and 7.0% respectively.
- No significant cross reactivity with human Apo AI, Apo AII, Apo CI, Apo CIII or Apo E.
- 10% cross reactivity with human Apo B.

Linearity

	Average Percentage of Expected Value		
Sample Dilution	**Plasma**	**Serum**	**Milk**
1:4000	94%	93%	96%
1:8000	100%	99%	99%
1:16000	110%	110%	101%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:2	93%
1:4	101%
1:8	103%

Recovery

Standard Added Value	2.5 – 25 ng/ml
Recovery %	83 – 112 %
Average Recovery %	98%

Plasma reference value: 78 -178 μg/ml.

Cross-Reactivity

Species	% Cross Reactivity
Canine	0.1%
Bovine	0.1%
Monkey	20%
Mouse	0.1%
Rat	0.1%
Swine	0.1%
Rabbit	0.1%
Human	100%

References

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