Product information

User’s Manual

Lipoprotein (a)
Lp(a) ELISA
Enzyme Immunoassay for the Determination of Lipoprotein a [Lp(a)] in Human Serum, Citrate and EDTA Plasma

IVD

REF DEM-DE7700040
1. INTRODUCTION

Lipoprotein(a) [Lp(a)] is a genetically determined and independent risk factor for atherosclerosis. Little is known about physiological function and metabolism of Lp(a). The serum concentration of this lipoprotein may vary between 0-200 mg/dl. At concentrations exceeding 30 mg/dl the risk for the development of atherosclerosis is markedly increased, particularly when there is a synchronous increase of the LDL-cholesterol.

The structure of Lp(a) is derived from the structure of the LDL particle. Lp(a) is a LDL particle, which is bound to the highly glycosylated apolipoprotein(a) [apo(a)] through one or more disulfide bridges. Homologies in the sequences of apo(a) and plasminogen indicate a correlation between thrombotic and atherosclerotic processes.

The protein and lipid composition of Lp(a) is characterized by inter- as well as intraindividual heterogeneity. More than 30 isoforms have been characterized so far. It is not known whether this heterogeneity influences the relative risk for the development of atherosclerosis.

In contrast to other lipoproteins, the concentrations of Lp(a) cannot be influenced by dietary measures. Lipid lowering drugs, which reduce the LDL concentration, have no effect on Lp(a).

2. INDICATION

Determination of a risk factor concerning Arteriosclerosis especially for patients with coronary heart diseases and myocardial infarct.

3. TEST PRINCIPLE

DEMEDITEC Lp(a) is a one-step sandwich ELISA. The test wells of the ELISA test strips are coated with specific, polyclonal anti-apo(a) antibodies. In a first incubation step diluted samples are incubated together with the conjugate (sample incubation). The conjugate consists of a specific, monovalent anti-apo(a)Fab-fragment coupled with peroxidase (anti-apo(a) peroxidase conjugate). During the incubation time the Lp(a) particles are bound to the solid phase and simultaneously marked by the conjugate. Unspecific serum components and unbound conjugate are removed by washing. In a second incubation step (substrate reaction) the enzyme reaction takes place. The peroxidase is part of the conjugate and oxidizes the substrate tetramethylbenzidine (TMB) to a blue coloured substance. To stop the reaction sulfuric acid is added and the colour changes to yellow. The colour intensity is directly proportional to the Lp(a) concentration in the sample. Optical density is measured at a wavelength of 450 nm by means of an ELISA reader. The Lp(a) concentration in the sample is quantitatively determined from the reference curve, which is run at the same time.

4. MATERIALS AND REAGENTS REQUIRED BUT NOT PROVIDED

- Distilled water
- Tubes for dilution of samples
- Graduated cylinder (1000 ml)
- Precision pipette (5 µl, 10 µl, 200 µl, 500 µl, and 1000 µl)
- Pipettes (10 ml and 20 ml)
- Multichannel pipette (50 µl, and 250 µl)
- Sample mixer
- Timer
- ELISA reader, 450 nm filter
5. MATERIALS AND REAGENTS PROVIDED

**MTP**, 12 ELISA test strips, with 8 wells each coated with affinity-purified, specific, polyclonal anti-apo(a) antibody from sheep; sealed in an aluminium bag with desiccant.  
*Ready to use!*

**Buffer concentrate (10x)**
0.4 M Tris/HCl, pH 8.4, contains detergent and protein stabiliser, 0.01% thimerosal, 2 bottles, 100 ml each.  
*Dilute before use!*

**Calibrators 1-5**; human sera with stabilizers and preservatives; 1 bottle each; lyophilized.  
*Concentrations are lot-specific as indicated on the bottle labels.*  
*Reconstitute before use!*

**POS LL, POS HL**
Positive control sera, positive; Pos LL, “Low Level”; Pos HL, “High Level”, for testing accuracy; human sera with stabilizers and preservatives; 1 bottle each; lyophilized.  
*Concentrations are lot-specific as indicated on the bottle labels.*  
*Reconstitute before use!*

**Conjugate**
anti-apo(a) peroxidase, polyclonal, specific, monovalent Fab-fragments from sheep, lyophilized, colored blue, 1 bottle.  
*Reconstitute before use!*

**Substrate TMB (tetramethylbenzidine) in methyprrolidone**
2 x 12 ml.  
*Ready to use!*

**Stop solution**
1 N acidic solution, 1 bottle containing 15 ml.  
*Ready to use!*

**Adhesive foil** for covering ELISA test strips, 2 pieces.

**Evaluation sheet** for plotting reference curve, 2 pieces.

**Patient protocol**, 1 piece.
6. TEST PERFORMANCE

6.1. Sample Material and Sample Storage
Human serum, citrated or EDTA plasma. Samples should be fresh. At -20°C they can be stored for several months. Samples may not be freezing/thawing several times.

Please note: Elevation of Ca2+ concentration and repeated freeze/thaw might result in aggregation of LDL-particles thus influencing the Lp(a) values.

6.2. Preparations

Before starting the test, bring all the required components to room temperature (20-26°C).

Preparation of working buffer, 1+9:
Example; add 100 ml buffer concentrate (10x) to 900 ml distilled water. Mix thoroughly!

Reconstitution of calibrators 1-5, and positive control sera:
Reconstitute calibrators 1-5 and control sera each with 200 µl working buffer for 15 min and mix for 10 sec (sample mixer). After reconstitution calibrators and control sera are clear or slightly turbid.

Dilution of calibrators, control sera and samples, 1+2000:
To obtain reliable test results it is very important to mix the dilutions well. The preparation of a predilution allows most exact pipetting.

Example 1:
Dilute 5 µl calibrators, 5 µl control sera or 5 µl samples with 10 ml working buffer.
Mix carefully for 10 sec (sample mixer)!

Example 2:
Predilution (1+100); dilute 10 µl calibrators, 10 µl control sera or 10 µl samples with 1000 µl working buffer. Final dilution (1+2000); dilute 50 µl predilution with 950 µl working buffer. Mix carefully!

Preparation of conjugate stock solution:
Reconstitute lyophilised conjugate with 1.25 ml working buffer. Mix it gently after 15 min (coloured blue).

Preparation of ready to use conjugate solution, 1+10:
Example for 8 wells; add 100 µl conjugate stock solution to 1000 µl working buffer. Prepare immediately before needed! The diluted conjugate is stable at room temperature (20-26°C) for 60 min.

6.3. Stability

Store the test kit and components at 2-8°C. The unopened reagents are stable until the expiry date indicated on the label.

Stability after opening:
- 6 month at 2-8°C (MTP in aluminium bag with desiccant): Substrate TMB, MTP, Buffer concentrate 10x
- 2 month at 2–8°C: ready-to-use working buffer
- 2 weeks at 2–8°C or 6 month at -20°C: reconstituted calibrators 1-5 and control sera
- 1 week at 2–8°C or 6 months at -20°C: conjugate stock solution
6.4. Test procedure

Sample and conjugate incubation:
Pipette **100 µl ready to use conjugate solution** into each test well needed and additionally pipette **100 µl diluted calibrators** (strips 1 and 2), diluted **controls** or **samples** into the wells. Cover test strips with adhesive foil and incubate at room temperature (20-26°C) for 120 min.

Wash:
Empty microassay strips and fill each well with **250 µl working buffer**. Empty wells again and repeat this wash step twice. Remove excess liquid by tapping the strips onto absorbent paper.

Substrate reaction:
Pipette **200 µl** ready to use substrate per well, incubate at **room temperature (20-26°C) for 30 min**.

Stop reaction:
Pipette 50 µl of stop solution per well, shake for 10 sec, measure colour within 10 min at 450 nm (reference wavelength at 650 nm).

7. NOTES FOR USER

- For professional use.
- Do not combine reagents of different lots.

Precision and recovery depend on following critical factors:
- Hemolytic, lipemic, icteric or microbial contaminated samples could cause false results.
- Perform the incubations at room temperature (20-26°C).
- Maintain an exact pipetting sequence.
- Run tests in duplicate.
- Incubation periods should not be exceeded by more than ±10%. Incubation period starts after the last pipetting step.
- The time to pipette the samples should not exceed 60 sec for each ELISA test strip.
- The time to pipette conjugate, substrate and stop solution should not exceed 10 sec for each ELISA test strip.

Security notes:
Stop solution (acidic solution) and components of substrate (methylpyrrolidone) may cause skin irritations. If acid or substrate should come into contact with eyes, rinse out immediately with plenty of water and consult a physician!
The human plasma used in this product has been tested for Human Immunodeficiency Virus (HIV 1 + 2), Hepatitis B and C and found to be negative (not repeatedly reactive).
However, all human blood products should be considered to be potentially infectious. Observe universal precautions concerning the handling of potentially infectious material.
Some of the reagents contain preservatives (e. g. thimerosal). Do not swallow! Avoid any contact with skin or mucous membranes!
Safety data sheet is available on request!
Disposal considerations
Product: Chemicals must be disposed of in compliance with the respective national regulations. Disposal of biological components should be in accordance with existing disposal practices employed for patient serum samples or infectious waste. Packaging: Packaging must be disposed of in compliance with the country-specific regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified differently, non-contaminated packaging may be treated like household waste or recycled.

Measures after damage on transport
If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use considerable damaged components for a test procedure. Store such components or kits until the complaint is handled.

8. EVALUATION

• Establishing the Reference Curve
• Use evaluation sheets provided in the pack.
  x-axis: Lp(a) Concentration in [mg/dl]
  y-axis: Absorbance (optical density) at 450 nm
• Use mean values of calibrators, control sera and sample measurements each.
• Plot the mean values of calibrators on the evaluation sheet and connect points with a curve ruler. If evaluation software is used, a programme for multiple and non-linear regression (e.g. 4-parametric curve adaption, Eq. 1) is recommended.

Eq. 1: \( Y = d + \frac{(a-d)}{1 + (\frac{x}{c})^b} \)

8.1. Quality Control
The extinction of the highest calibrator should be between 1.5 and 2.5. The absorbance of calibrator 1 should not exceed 0.5.
The difference in the absorbance of calibrators 5 and 1 should be at least 1.0.
The concentration of the control sera may be taken from the reference curve. The control levels obtained are used to check whether evaluation is correct.

8.2. Determination of Sample Concentration
Concentration of the samples may be taken from the reference curve. If the sample absorbance exceeds the absorbance of calibrator 5, the sample should be prediluted with the working buffer (1+1). The concentration thus obtained has to be multiplied by the dilution factor 2.
The conversion of citrated plasma to serum values is achieved by multiplying the recorded concentrations with the factor 1.1.
9. INTERPRETATION OF THE TEST RESULTS

The interpretation of values obtained is influenced by genetic factors (e.g., polymorphism, gender-specific and ethnic differences) and by the asymmetric incidence distribution of Lp(a) values: e.g., the median value for Caucasians approximates at 10 mg/dL, for Chinese at 7 mg/dL, and for Sudanese at 40 mg/dL. For Caucasians a 15-20% risk to develop atherosclerotic symptoms has been described for individuals with plasma levels of 25-30 mg/dL.

Test results can be interpreted according to the table below:

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Lp(a) [mg/dL]</th>
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<tbody>
<tr>
<td>Normal range</td>
<td>&lt;25</td>
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<tr>
<td>Elevated risk / boundary</td>
<td>25-35</td>
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<tr>
<td>Pathologic range</td>
<td>&gt;35</td>
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10. TEST CHARACTERISTICS

**Intraassay variation:**
Four samples in the concentration range 18-55 mg/dL were measured each 15 times in one run in double determination. The intraassay variation related to the concentration values ranged between 4% and 6%.

**Interassay variation:**
Three series with 3 samples (19.7 mg/dL, 34.6 mg/dL, and 59.6 mg/dL) were measured with a lot in double determination. The interassay variation related to the concentration values ranged between 7% and 9%.

**Sensitivity:**
The detection limit of the test is <5 mg/dL.

**Specificity of immune reaction:**
Cross reactions with plasminogen and LDL are below the limit of detection. The antibodies used in this assay identify all known isoforms of apo(a).

**Standardisation:**
There is no established method for the determination of Lp(a) and no established standard. However, values obtained with the DEMEDITEC Lp(a) showed a very good correlation with values obtained by turbidimetric methods (see figure 2).

![Figure 2: Correlation curve ELISA/LEIA](image-url)
11. REFERENCES


### SYMBOLS USED WITH DEMEDITEC ASSAYS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>Deutsch</th>
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