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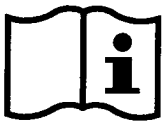

Userś Manual

Lipoprotein (a) ELISA

Enzyme Immunoassay for the quantitative determination of Lipoprotein a [Lp(a)] in human serum, citrate and EDTA plasma

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1. INTENDED USE

Enzyme immunoassay for the quantitative determination of Lipoprotein a [Lp(a)] in human serum, citrate and EDTA plasma.

2. SUMMARY AND EXPLANATION

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 disulphide 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).

3. TEST PRINCIPLE

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 peroxidise (anti-apo(a) peroxidise 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 peroxidise is part of the conjugate and oxidizes the substrate tetramethylbenzidine (TMB) to a blue coloured substance. To stop the reaction acidic solution 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 my means of a ELISA reader. The Lp(a) concentration in the sample is quantitatively detemined from the reference curve, which is run at the same time.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact Demeditec or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available upon request directly from Demeditec.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters. The microtiter strips, the TMB Substrate and the Buffer are stable **up to 6 months in the broken**, but tightly closed (MTP in aluminium bag with desiccant) when stored at 2–8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA, Citrate)

Samples should be fresh. At -20 °C they can be stored for several months. Samples may not be freezing/thawing several times. The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

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

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
12 x 8	**SORB MT**	**Microtiter Plate** Ready to use. Break apart strips. Coated with affinity-purified, specific, polyclonal anti-apo(a) antibody from sheep.
1 x 1.3 mL	**ENZ CONJ**	**Enzyme Conjugate** lyophilized. Blue colored. Contains: anti-apo(a) peroxidase, polyclonal, specific, monovalent Fab-fragments from sheep.
5 x 0.2 mL	**CAL 1-5**	**Calibrators** lyophilized. Contains: Human serum, stabilizers, preservatives. For exact concentrations see QC certificate.
2 x 0.2 mL	**CONTROL**	**Control 1+2** lyophilized. Positive Control Serum, 1, “Low Level” and 2, “High Level” Contains: Human serum, stabilizers, preservatives. Acceptable ranges see QC certificate.
2 x 100 mL	**BUF 10x**	**Wash Solution (Buffer)** Concentrate (10x) Contains: detergents, stabilizers, 0.01 % (w/v) Thimerosal, 0.4 M Tris/HCl; pH 8.4.
2 x 15 mL	**SUB TMB**	**TMB Substrate Solution** Ready to use. Contains: TMB (Tetramethylbenzidine).
1 x 15 mL	**STOP SOLN**	**TMB Stop Solution** Ready to use. Contains: 1 N acidic solution
2 x		**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices,<3% CV). Volume: 5, 50, 100, 200, 1000 µL
2. Vortex mixer
3. Tubes for sample dilution
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of concentrated components

Dilute / dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
100 mL	**BUF 10x**	900 mL	bidist. water	1:10	Mix thoroughly.	2-8 °C	2 months

10.2. Preparation of lyophilized components

Dilute / dissolve	Component	with	Diluent	Remarks	Storage	Stability
1 vial	**CAL 1-5** **Control 1** **Control 2**	200 µL	**Buffer (diluted)**	Leave for 15 min at room temperature (18 - 25 °C) and mix 10 seconds on sample mixer (avoid generation of foam).	2-8 °C	2 weeks
					-20 °C	6 months
1 vial	**ENZ CONJ**	1.30 mL	**Buffer (diluted)**		2-8 °C	1 week
					-20 °C	6 months

After reconstitution calibrators and control sera are clear or slightly turbid.

10.3. Dilution of Standards, Controls and Samples

	to be diluted	with	Relation	Remarks
CAL 1-5 **Control 1** **Control 2**	generally	**Buffer (diluted)**	1:2001	e.g. 5 µL **CAL** + 10 mL **BUF 10x** e.g. 5 µL **Control** + 10 mL **BUF 10x**
Serum, Plasma	generally	**Buffer (diluted)**	1:2001	e.g. 5 µL + 10 mL **BUF 10x**
ENZ CONJ	generally	**Buffer (diluted)**	1:11	e.g. 400 µL **ENZ CONJ** + 4 mL **BUF 10x** Stability: 60 min (18-25 °C).

11. TEST PROCEDURE

1.	Pipette **100 µL** of **Conjugate solution** into each well needed and additionally pipette **100 µL,** diluted **calibrators,** diluted **controls or samples** into the respective wells of the Microtiter plate (Calibrators should be placed in strips 1 and 2).
2.	Cover plate with adhesive foil. **Incubate 120 min** at **RT (18-25°C)**.
3.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **250 µL** of **diluted Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
4.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution.
5.	Pipette **200 µL** of **TMB Substrate Solution** into each well.
6.	**Incubate 30 min** at **RT (18-25°C).**
7.	Stop the substrate reaction by adding **50 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
8.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600 - 650 nm) within **10 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

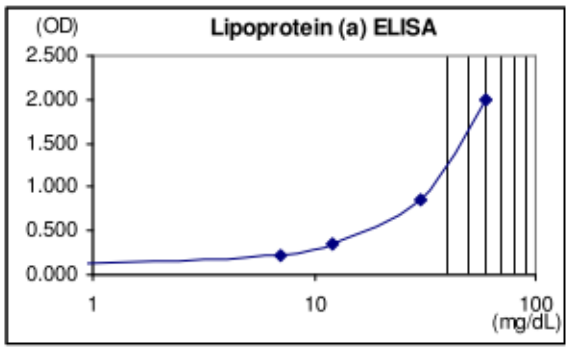
13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log. For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read directly from the standard curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed. The conversion of citrated plasma to serum values is achieved by multiplying the recorded concentrations with the factor 1.1.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	mg/dL	OD_{Mean}
CAL 1	0	0.016
CAL 2	7	0.222
CAL 3	12	0.359
CAL 4	30	0.851
CAL 5	60	2.005



14. INTERPRETATION OF 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:

Assessment	Lp(a) [mg/dL]
Normal range	<25
Elevated risk / boundary	25-35
Pathologic range	>35

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests. It is recommended that each laboratory establishes its own range of normal values.

15. PERFORMANCE

Analytical Specificity (Cross Reactivity)	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).		
Precision	Range (mg/dL)		CV Range (%)
Intra-Assay	18 – 55		4 – 6
Inter-Assay	19.7 – 59.6		7 – 9
Linearity	Dilution	Measured (mg/dL)	Linearity (%)
	1:1	35.21	100
	1:2	31.86	90
	1:4	35.83	102
	1:8	24.71	70
	1:1	31.31	100
	1:2	29.24	93
	1:4	31.14	99
	1:8	25.05	80
	1:1	34.99	100
	1:2	35.36	101
	1:4	37.80	108
	1:8	27.55	79
Analytical Sensitivity	<5 mg/dL		
Method Comparison	Demeditec-ELISA = 4.322 + 1.052 x LEIA		r = 0.959

Standardisation: There is no established method for the determination of Lp(a) and no established standard.
Correlation: Values obtained with the Lipoprotein (a) ELISA showed a very good correlation with values obtained by turbidimetric methods (see figure 2).

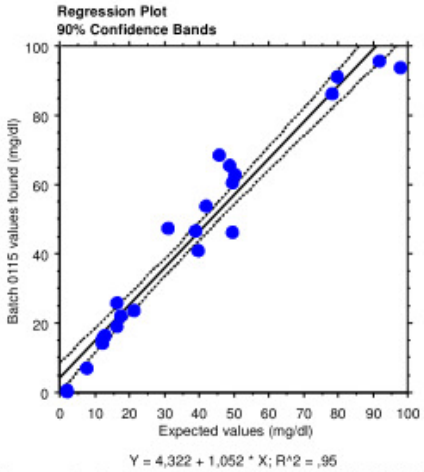


Figure 2: Correlation curve ELISA/LEIA

16. PRODUCT LITERATURE REFERENCES

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SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
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Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
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