Mercodia Lp(a) ELISA

Directions for Use

10-1106-01
Reagents for 96 determinations

For *in vitro* diagnostic use in EU/EEA, UK , US and Canada

IVD

CE

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Regulatory status in the rest of the world: For research use only.
Not for use in diagnostic procedures.

Manufactured by

Mercodia AB
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Sweden

Explanation of symbols used on labels

	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
	Lot No.
	For *in vitro* diagnostic use

Intended use

Mercodia Lp(a) ELISA provides a method for the quantitative determination of human Lp(a) in serum or plasma.

Summary and explanation of the test

Apolipoprotein(a), Apo(a), is a glycoprotein linked by disulphide bridges to apolipoprotein B in the Lp(a) particle. Apo(a) is formed by three different structural domains. One of the domains, called kringle 4, type 2, is present in multiple copies, the number of which varies and is genetically determined, giving rise to different sizes of Apo(a). Depending on the method used, six to 23 different isoforms of Apo(a) ranging from about 300 to 900 kD have been identified [1,2,15,16]. Most individuals have two Apo(a) isoforms, although in some subjects no Apo(a) band can be detected when analyzed in SDS-gel electrophoresis followed by immunoblotting [3].

Recently, much interest has been focused on Lp(a) since there is a lot of evidence that circulating levels represents an independent risk factor for coronary vascular disease. The Lp(a) level has been found to be an inherited risk factor for ischaemic heart disease [4-8]. High Lp(a) levels have been demonstrated in familial hypercholesterolemia and its measurement may be clinically useful for risk prediction in these patients [9,10].

Results have also been published on Lp(a) as a strong indicator for cerebrovascular disease [11,12].

Apo(a) is homologous to the protease zymogen plasminogen [13,14]. Lp(a) inhibits plasminogen activation and recent studies have shown that Apo(a) compete with plasminogen for binding to the plasminogen receptor. These properties of Apo(a) may explain the association of high Lp(a) concentrations with myocardial infarction.

Principle of the procedure

Mercodia Lp(a) ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the Apo(a) molecule. During incubation Apo(a) in the sample react with per-oxidase-conjugated anti-Apo(a) antibodies and anti-Apo(a) antibodies bound to microtitration well. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

Warnings and precautions

- For in vitro diagnostic use in EU/EEA, UK , US and Canada.
- Regulatory status in the rest of the world: For research use only. Not for use in diagnostic procedures.
- All samples should be handled as of capable of transmitting infections.
- Each well can only be used once.
- The Stop Solution contains <5% Sulphuric acid.
  The Stop Solution is labeled:

Danger

H318 – Causes serious eye damage.
H315 – Causes skin irritation.
P280 – Wear protective gloves. Wear eye or face protection.
P264 – Wash hands thoroughly after handling.
P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.
P332 + P313 - If skin irritation occurs: Get medical attention.
P305 + P351 + P338 + P310 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.Immediately call a POISON CENTER or physician.

- The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4, Wash Buffer and Sample Buffer contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one. (3:1)

  The Enzyme Conjugate Buffer, the Calibrators, Wash Buffer and Sample Buffer are labeled:

Warning

H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

- The Pretreatment Solution contains <10% Tri-Sodium phosphate dodecahydrate.
  The Pretreatment Solution is labeled:

Warning

H319 – Causes serious eye irritation.
H315 – Causes skin irritation.
P280 – Wear protective gloves. Wear eye or face protection.
P264 – Wash hands thoroughly after handling.
P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.
P332 + P313 - If skin irritation occurs: Get medical attention.
P305 + P351 + P338 + P310 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

Warning! This kit contains reagents that may be infectious!
This kit contains reagents manufactured from human blood components. The source of material have been tested by immunoassay for hepatitis B surface antigen, antibodies for Hepatitis C virus and antibodies for HIV virus and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivates should be observed. Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/national guide-lines on laboratory saftey procedures.

Material required but not provided

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader with 450 nm filter
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

Reagents

Each Mercodia Lp(a) ELISA kit contains reagents for 96 wells, sufficient for 43 samples and one Calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2–8°C.

Coated Plate Mouse monclonal anti-Apo(a)	1 plate	96 wells 8-well strips	Ready for use
For unused microplate wells completely reseal the bag by using adhesive tape. Store at 2-8°C, use within 2 months.			
Calibrators 1, 2, 3, 4 Human Lp(a) Concentration indicated on vial label. Color coded yellow	4 vials	500 µL	Lyophilized Add 500 µL redist. water per vial.
For storage of reconstituted Calibrators for more than 1 week, store at –20°C.			
Calibrator 0 Color coded yellow	1 vial	500 µL	Ready for use
Enzyme Conjugate 11X Peroxidase conjugated mouse monoclonal anti-Apo(a)	1 vial	700 µL	Preparation, see below.
Enzyme Conjugate Buffer Color coded blue	1 vial	7 mL	Ready for use
Pretreatment Solution	1 vial	5 mL	Ready for use
Sample Buffer 5X Color coded red	2 bottles	50 mL	
Dilute each bottle with 200 mL redistilled water to make sample buffer 1X solution. *Note!* Precipitate may occur when stored at 2-8°C. Allow Sample Buffer 5X to reach room temperature. Mix until precipitate has dissolved.			
Wash Buffer 21X Storage after dilution: 2-8°C for 2 months	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution.
Substrate TMB Colorless solution *Note! Light sensitive!*	1 vial	22 mL	Ready for use
Stop Solution 0.5 M H_2SO_4	1 vial	7 mL	Ready for use

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by mixing Enzyme Conjugate 11X in Enzyme Conjugate Buffer (1+10) according to the table. When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently. Store at 2-8°C. Use within 2 weeks.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
6 strips	300 µL	3.0 mL
4 strips	200 µL	2.0 mL

Specimen collection and handling

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Specimen may be stored for 1 week at 2-8°C. For longer periods store samples at -20°C. Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing EDTA or heparin as anticoagulant, and separate the plasma fraction by centrifugation. Specimen may be stored for 1 week at 2-8°C. For longer periods store samples at -20°C. Avoid repeated freezing and thawing.

Preparation of samples

All samples have to be pretreated as follows:

1	Sample	25 µL
2	Pretreatment Solution	25 µL
3	Mix and incubate for 1 hour at room temperature	
4	Add sample buffer and mix	5.0 mL

As a result of this procedure the samples will be diluted 1/202. This dilution is stable for 1 week at 2–8°C.

If the concentration of Lp(a) in the sample is >1000 U/L, dilute the pretreated and diluted sample (1/202) further in sample buffer, e.g. 1/4 giving a final dilution of 1/808.

Test procedure

Prepare enzyme conjugate 1X solution, wash buffer 1X solution and sample buffer 1X solution. Perform each determination in duplicate for Calibrators, controls and samples. Prepare a calibrator curve for each assay run. Avoid pipetting solution onto the walls. The product has been optimized and validated without plate sealer.

	Add to anti-Lp(a) wells	Calibrators	Samples
1.	Calibrators	25 µL	–
2.	Pretreated samples/controls	–	25 µL
3.	Enzyme conjugate 1X solution	50 µL	50 µL

4. Incubate on a shaker (700-900 rpm) for 1 hour at room temperature (18–25°C).
5. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function, after final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.
   Or manually,
   discard the reaction volume by inverting the microplate over a sink.
   Add 350 µL wash buffer 1X solution to each well. Discard the wash buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
6. Add 200 µL Substrate TMB.
7. Incubate for 15 minutes.
8. Add 50 µL Stop Solution.
   Put the plate on the shaker for 5 seconds to ensure mixing of Substrate and Stop Solution.
9. Measure the absorbance at 450 nm and evaluate.
   Read within 30 minutes.

Note! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution

Internal quality control

Internal plasma pools with low, intermediate and high Lp(a) concentration should routinely be assayed as samples, and results charted from day to day, it is good laboratory practice to record the following data for each assay: kit lot number; reconstitution dates of kit components; OD values for the blank and Calibrators.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

Calculations of results

The concentration of Lp(a) is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the Lp(a) concentration using cubic spline regression. Multiply the concentration of the samples with the dilution factor (e.g. × 202).

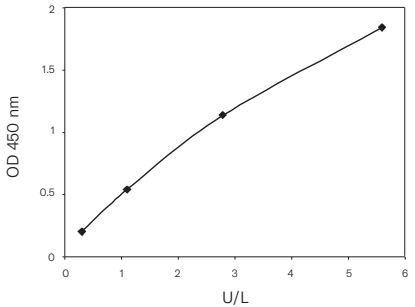
Example of worksheet

Wells	Identity	$A_{450\ nm}$	Mean conc. U/L*
1A–B	Calibrator 0	0.061/0.064	
1C–D	Calibrator 1**	0.194/0.197	
1E–F	Calibrator 2**	0.535/0.537	
1G–H	Calibrator 3**	1.129/1.131	
2A–B	Calibrator 4**	1.835/1.837	
2C–D	Sample 1	0.286/0.286	104.5
2E–F	Sample 2	0.562/0.563	238.4
2G–H	Sample 3	1.070/1.073	525.4

*Result multiplied by dilution factor (x 202).
**Concentration indicated on vial label.

Example of calibrator curve

A typical calibrator curve is shown below. Do not use this curve to determine actual assay results.



Limitations of the procedure

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.

Grossly lipemic, icteric or hemolysed samples do not interfere in the assay.

Expected values

Good practice dictates that each laboratory establishes its own expected range of values.

Performance characteristics

Detection limit

Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured. The detection limit is 0.07 U/L as determined by the methodology described in ISO11843-Part 4. Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition is 96–111 % (mean 102 %).

Hook effect

Samples with a Lp(a) concentration of up to 9600 U/L can be measured without giving falsely low results if they are pretreated and diluted 1/202 as described above.

Precision

Samples pretreated and diluted 1/202 on one occasion and stored at –20°C until the assays were performed. Each sample was analyzed in 4 replicates on nine different occasions.

		Coefficient of variation	
Sample	Mean value U/L	Repeatability %*	Within laboratory %**
1	83	3.3	5.2
2	196	2.9	4.7
3	485	2.4	3.0

*Within assay variation
**Total assay variation

Samples pretreated and diluted 1/202 on each test occasion. Each sample was analyzed in 5 replicates on five different occasions.

		Coefficient of variation	
Sample	Mean value U/L	Repeatability %*	Within laboratory %**
1	103	3.1	5.2
2	251	3.6	5.2
3	744	2.4	5.7

*Within assay variation
**Total assay variation

Specificity

A concentration of up to 10 g/L of plasminogen gives no measurable cross-reactivity in the assay (Clinical concentration of plasminogen is below 2.1 g/L).

Apolipoprotein B has no measurable cross-reactivity.

Calibration

Mercodia Lp(a) ELISA kit is calibrated against a highly purified, fully validated, commercial Lp(a) preparation.

The concentration of the Mercodia Lp(a) ELISA is expressed in Units/L.

Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorised distributors, in such event, shall not be liable for damages indirect or consequential.

References

1. Utermann G. (1989) The mysteries of lipoprotein (a). *Science* 17 Nov:904–910
2. MBewu AD and Durrington PN (1990) Lipoprotein (a): structure, properties and possible involvement in trombogenesis and atherogenesis. *Atherosclerosis* 85:1–14
3. Albers JJ, Marcovina SM and Lodge MS (1990) The unique lipoprotein(a): properties and immunochemical measurement. *Clin Chem* 36: 2019–2026
4. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B and Wendel H (1990) Lipoprotein(a) and coronary heart disease: a prospective case control study in a general population sample of middle aged men. *Br Med J* 301:1248–1251
5. Rhoads GG, Dahlén G, Berg K, Morton NE and Danneberg AL (1986) Lp(a) Lipoprotein as a risk factor for myocardial infarction. *JAMA* 256:2540–2544
6. Dahlen GH, Guyton JR, Attar M, Farmer JA, Kautz JA and Gotto AM Jr (1986) Association levels of lipoprotein Lp(a), plasma lipids and other lipoproteins with coronary artery disease documented by angiography. *Circulation* 74:758–765
7. Dembinski T, Nixon P, Shen G, Mymin D and Choy PC. (2000) Evaluation of a new apolipoprotein(a) isoform-independent assay for serum Lipoprotein(a). *Mol Cell Biochem* 207:149–155
8. Houlston R and Friedl W (1988) Biochemistry and clinical significance of lipoprotein (a). *Ann Clin Biochem* 25:499–503
9. Wiklund O, Angelin B, Olofsson SO, Eriksson M, Fager G, Berglund L and Bondjers G (1990) Apolipoprotein (a) and ischaemic heart disease in familial hypercholesterolaemia. *Lancet* 335:1360–1363
10. Seed M, Hoppichler F, Reaveley D, McCarthy S, Thopmson GR, Boerwinkel E and Utermann G (1990) Relation of serum lipoprotein(a) concentration and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia. *New En J of Med* 322:1494–1499
11. Zenker G, Költringer P, Boné G, Niederkorn K, Pfeiffer K and Jürgens G (1986) Lipoprotein(a) as a strong indicator for cerebrovascular disease. *Stroke* 17:942–945
12. Murai A, Miyahara T, Fujimoto N, Matsuda M and Kameyama M (1986) Lp(a) lipoprotein as a riskfactor for coronary heart disease and cerebral infarction. *Atherosclerosis* 59:199–204
13. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM and Lawn RM (1987) cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 330: 132–137
14. Eaton DL, Fless GM, Kohr WJ, McLean JW, Xu QT, Miller CG, Lawn RM and Scanu AM(1987) Partial amino acid sequence of apolipoprotein (a) shows that it is homologous to plasminogen *Biochemistry* 84:3224–3228

15. Lackner C, Boerwinkle E, Leffert CC, Rahmig T and Hobbs HH (1991) Molecular basis of apolipoprotein(a) isoform size heterogenity as revealed by pulsed-field gel electrophoresis. *J Clin Invest* 87:2153–2161

16. Kamboh MI, Ferrell RE and Kottke BA (1991) Expressed hypervariable polymorphism of apolipoprotein (a). *Am J Hum Genet* 49:1063–1074

17. Solymoss BC, Marcil M, Wesolowska E, Gilfix BM, Lespérance J and Campeau L (1993) Relation of coronary artery disease in women <60 years of age to the combined elevation of serum lipoprotein(a) and total cholesterol to high-density cholestrolratio. *Am J Cardiol* 72:1215–19

Further references can be found on our website: **www.mercodia.com**

Notes

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment: **Date:** **KIT LOT#:**

Summary of protocol sheet
Mercodia Lp(a) ELISA

Add Calibrators and pretreated controls* and samples	25 µL
Add enzyme conjugate 1X solution	50 µL
Incubate	1 hour at 18–25°C on a plate shaker (700–900 rpm)
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add Substrate TMB	200 µL
Incubate	15 minutes
Add Stop Solution	50 µL Shake for 5 sec to ensure mixing
Measure $A_{450\ nm}$	Evaluate results

*not included

For full details see page 8

For technical support please contact: support@mercodia.com

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