Mercodia
Developing diagnostics

Mercodia Lp(a) ELISA

Directions for Use

10-1106-01
REAGENTS FOR 96 DETERMINATIONS

For *in vitro* diagnostic use

IVD

CE

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Veuillez trouver le mode d'emploi en français à:
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Manufactured by

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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 peroxidase-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 PRECAUTION

- For *in vitro* diagnostic use.
- Not for internal or external use in humans or animals.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All patient specimens should be handled as if capable of transmitting infections.

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 for 25 µL, 50 µL, 200 µL, 500 µL and 5 mL (repeat pipettes preferred for addition of enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Test tubes, 5 mL
- Microplate reader with 450 nm filter
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device
- Magnetic stirrer

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 8-well strips	96 wells	Ready for use
For unused microplate wells completely reseal the bag by using adhesive tape. Store at 2-8°C, use within 8 weeks.			
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 one 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 Dilute with 1000 mL redistilled water to make wash buffer 1X solution. Storage after dilution: 2-8°C for 8 weeks	1 bottle	50 mL	
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.

Numbers 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. 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 and samples. Prepare a calibrator curve for each assay run. Avoid pipetting solution onto the walls.

Add to anti-Apo(a) wells	Calibrators	Samples
1 Calibrators	25 µL	–
2 Pretreated samples	–	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! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

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.

CALCULATIONS OF RESULTS

Computerized calculations

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)

Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except for Calibrator 0, against the Lp(a) concentration on a lin-lin paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.
3. Multiply the concentration of the samples with the dilution factor (e.g. x 202).

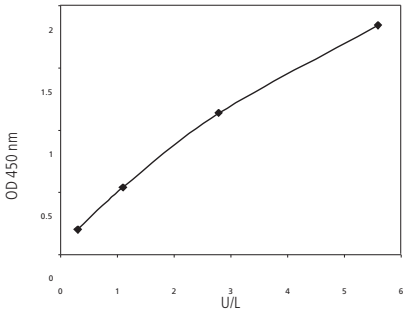
Example of worksheet

Wells	Identity	A_{450}	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 (× 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.



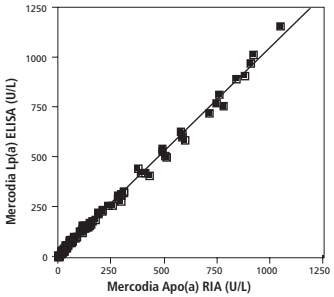
LIMITATIONS OF THE PROCEDURE

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.

COMPARISION WITH MERCODIA Apo(a) RIA

Comparison studies between Mercodia Lp(a) ELISA and Mercodia Apo(a) RIA have been performed with 45 samples assayed in 2 replicates on 2 occasions. The values found, show a good correlation between the two techniques, r=1.00 (see figure). Thus, the expected values for Mercodia Apo(a) RIA can be used for Mercodia Lp(a) ELISA as well.



EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values. The following results obtained with Mercodia Apo(a) RIA may serve as a guide until the laboratory has gathered sufficient data of its own.

The Lp(a) level has been studied in three different materials:

A Normals, n=171, Sweden (Caucasian).
B Normals, n=203, Canada (Caucasian-Asian, heterogeneous).
C Patients with familial hypercholesterolemia (FH), n=113, Canada (Caucasian-Asian, heterogeneous).

The group of normals were individuals chosen from the general population and with no apparent cardio and/or cerebrovascular disease.

The distribution is shown in the following figures.

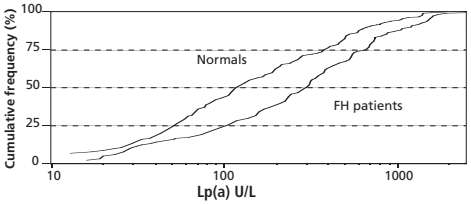
The three groups investigated did not show any age or sex differences in their Lp(a) levels. No significant difference in Lp(a) levels was found between the group of normals from Sweden and the group of normals from Canada.

The group of FH patients had significantly higher Lp(a) levels than the group of normals from the same region ($p<0.001$, Wilcoxon rank sum test).

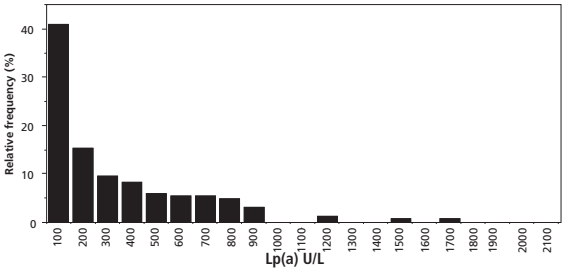
The following Lp(a) concentrations for median, 75th, 85th and 95th percentiles were obtained for the different groups.

	Median U/L	75th perc. U/L	85th perc. U/L	95th perc. U/L
Normals, Sweden	131	448	612	795
Normals, Canada	117	379	525	1044
FH, Canada	294	660	863	1544

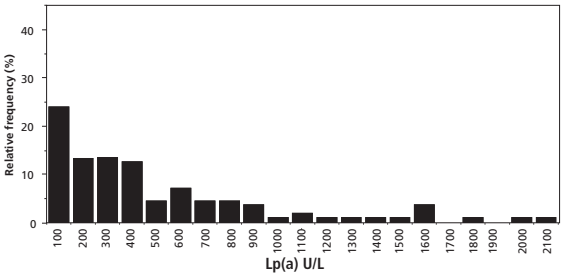
Lp(a) distribution in normals and FH patients (Canada)



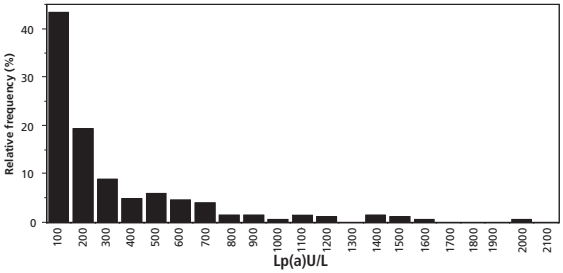
Distribution of normals (Canada)



Distribution of normals (Sweden)



Distribution of FH patients (Canada)



PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit is 0.05 U/L calculated as three standard deviations above the Calibrator 0. This corresponds to a sample concentration of 10 U/L when the sample is diluted 1/202.

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	Obtained value U/L	within assay %	between assay %	total assay %
1	83	3.3	4.0	5.2
2	196	2.9	3.6	4.7
3	485	2.4	1.8	3.0

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	Obtained value U/L	within assay %	between assay %	total assay %
1	103	3.1	4.2	5.2
2	251	3.6	3.7	5.2
3	744	2.4	5.2	5.7

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.

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SUMMARY OF PROTOCOL SHEET

Add Calibrators and pretreated samples	25 µL
Add enzyme conjugate 1X solution	50 µL
Incubate	1 hour at 18–25°C on a shaker
Wash	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}	

X-O Graf Tryckeri AB