

Mercodia Apo(a) ELISA

Directions for Use

Mode d'emploi

Istruzioni per l'uso

Bruksanvisning

Gebrauchsinformation

Instrucciones para el uso

Brugsanvisning

10-1106-01

REAGENTS FOR 96 DETERMINATIONS



Manufactured by/Hersteller/Fabriqué par/

Fabricado por/Prodotto da/Fremstillet af/

Tillverkad av

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Mercodia 

The Mercodia logo is a stylized grey triangle pointing to the right, with a small circle inside it.

EXPLANATION OF SYMBOLS USED ON LABELS/ERKLÄRUNG DER SYMBOLE AUF DEN ETIKETTEN/EXPLICATION DES SYMBOLES UTILISÉS SUR LES ÉTIQUETTES/EXPLICACIÓN DE LOS SÍMBOLOS UTILIZADOS EN LAS ETIQUETAS/SPIEGAZIONE DEI SIMBOLI USATI SULLE ETICHETTE/FORKLARING AF SYMBOLER ANVENDT PÅ ETIKETTER/FÖRKLARING AV SYMBOLERNA SOM ANVÄNDS PÅ ETIKETTERNA

 <p>$\Sigma = 96$</p>	<p>Reagents for 96 determinations Reagenzien für 96 Bestimmungen Réactifs pour 96 mesures Reactivos para 96 determinaciones Reagenti per 96 rilevazioni Reagens til 96 bestemmelser Reagenser för 96 bestämningar</p>
	<p>Expiry date Verfallsdatum A utiliser avant Fecha de caducidad Data di scadenza Udløbsdato Utgångsdatum</p>
	<p>Store between 2–8°C Lagerungstemperatur 2–8°C A conserver entre 2 et 8 °C Conservar a entre 2–8 °C Conservare tra i 2–8 °C Opbevar ved 2–8°C Förvara vid 2–8°C</p>
<div style="border: 1px solid black; padding: 5px; display: inline-block;"> <p>LOT</p> </div>	<p>Lot No. Lot Nr. N° de lot N° lote Lotto n. Partinr. Lotnr.</p>
<div style="border: 1px solid black; padding: 5px; display: inline-block;"> <p>IVD</p> </div>	<p>For <i>in vitro</i> diagnostic use Zum Gebrauch in der <i>in vitro</i>-Diagnose Ce kit est réservé à l'utilisation diagnostique <i>in vitro</i> Para uso diagnóstico <i>in vitro</i> Per l'uso diagnostico <i>in vitro</i> Til <i>in vitro</i>-diagnosticering För <i>in vitro</i> diagnostiskt bruk</p>

INTENDED USE

Mercodia Apo (a) ELISA provides a method for the quantitative measurement of human apolipoprotein (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 lipoprotein(a) (Lp(a)) particle. Apo(a) is formed by three different structural domains. One of the domains, called kringle 4, is present in multiple copies, the number of which varies and is genetically determined, giving rise to different sizes of apo(a) and consequently Lp(a). Depending on the method used, six to 23 isoforms of apo(a) ranging from about 300 to 900 kD have been identified (1,2,15,16). Most individuals have one or two Apo(a) isoforms, although in some subjects no Apo(a) band can be detected when analysed 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) and Lp(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 Apo(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 apolipoprotein(a) molecule. During incubation apolipoprotein(a) in the sample react with peroxidase-conjugated anti-apolipoprotein(a) antibodies and anti-apolipoprotein(a) antibodies bound to microtitration well. A simple washing step removes unbound enzyme labelled 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 H₂SO₄. 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 derivatives should be observed. Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/national guide-lines on laboratory safety procedures.

MATERIAL REQUIRED BUT NOT PROVIDED

- 25 µl and 500 µl micropipette with disposable tips
- 25 µl, 50 µl, 200 µl and 5.0 ml repeating pipettes
- 250 ml and 1000 ml beaker
- 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 Apo(a) ELISA kit contains reagents for 96 wells, sufficient for 41 samples, 2 Controls 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. For storage of reconstituted Calibrators and Controls for more than one week, store at –20°C. For unused microtitration strips, reseal the bag using adhesive tape and store at +2–8°C for two months.

Coated Plate (mouse monoclonal anti-Apo(a))	1 plate 8-well strips	96 wells	Ready for use
For unused microplate wells completely reseal the bag using adhesive tape and use within two months			
Calibrators 1, 2, 3, 4 (human Apo(a)) Concentration indicated on vial label	4 vials	500 µl	Add 500 µl redist. water per vial lyophilized
Calibrator 0 Color coded yellow	1 vial	500 µl	Ready for use
Enzyme Conjugate 11X (Peroxidase conjugated mouse monoclonal Anti-apo(a)) <i>Note! Light sensitive!</i>	1 vial	700 µl	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	7 ml	Ready for use
Controls (H), (L) Apo(a) concentration indicated on vial label (Mean ± 3 S.D.)	2 vials	500 µl	Add 500 µl redist. water per vial lyophilized
Pretreatment Solution	1 vial	5 ml	Ready for use
Sample Buffer 5X Color coded red Dilute each bottle with 200 ml redistilled water to make sample buffer. <i>Note! Precipitate may occur when stored at +2-8 °C. Allow Sample Buffer 5X to reach room temperature. Shake or vortex until precipitate has dissolved.</i>	2 bottles	50 ml	
Wash buffer 21X Storage after dilution: +2-8°C for 4 weeks	1 bottle	40 ml	Dilute 1+20 with 800 ml redist water to make wash buffer
Substrate TMB TMB Colorless solution <i>Note! Light sensitive!</i>	1 vial	22 ml	Ready for use
Stop Solution 0.5 M H ₂ SO ₄	1 vial	7 ml	Ready for use

Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate solution by mixing Enzyme Conjugate 11X in Enzyme Conjugate Buffer (1+10) according to the table. When preparing enzyme conjugate solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently. Use within 2 weeks at +4°C.

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 serum samples and Controls have to be pretreated as follows:

1 Serum sample/Control	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 apolipoprotein(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 solution, wash buffer and sample buffer. Perform each determination in duplicate for Calibrators, Controls and unknowns. Prepare a calibrator curve for each assay run. Avoid pipetting solution onto the walls.

Add to anti-Apo (a) wells	Calibrators	Unknowns	Controls
1 Calibrators	25 μ l	–	–
2 Pretreated samples	–	25 μ l	–
3 Pretreated Controls	–	–	25 μ l
4 Enzyme conjugate solution	50 μ l	50 μ l	50 μ l
5 Incubate on a shaker for 1 hour at room temperature (18–25 °C).			
6 Wash plate 6 times with automatic plate washer or Aspirate the reaction volume. Add 350 μ l wash buffer to each well. Aspirate completely. Repeat 5 times. After final wash, invert and tap the plate firmly against absorbent paper.			
7 Add 200 μ l Substrate TMB			
8 Incubate for 15 minutes			
9 Add 50 μ l Stop Solution. Put the plate on the shaker for 15 seconds to ensure mixing of Substrate and Stop Solution.			
10 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 Apo(a) concentration should routinely be assayed as unknowns, 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, calibrators and Controls.

CALCULATIONS OF RESULTS

Computerized calculations

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

Manual calculation

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

Example of worksheet

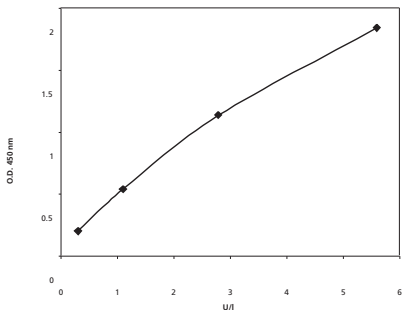
Values obtained with an 8 weeks old kit.

Wells	Identity	A ₄₅₀	Mean conc. U/l*
1A–B	Calibrator 0	0.061/0.064	
1C–D	Calibrator 0.31 U/l	0.194/0.197	62.6
1E–F	Calibrator 1.11 U/l	0.535/0.537	224.2
1G–H	Calibrator 2.8 U/l	1.129/1.131	565.6
2A–B	Calibrator 5.6 U/l	1.835/1.837	1131.2
2C–D	Control L	0.239/0.242	83.8
2E–F	Control H	0.515/0.515	215.4
2G–H	Unknown 1	0.286/0.286	104.5
3A–B	Unknown 2	0.562/0.563	238.4
3C–D	Unknown 3	1.070/1.073	525.4

* Result multiplied by dilution factor ($\times 202$).

Example of calibrator curve

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



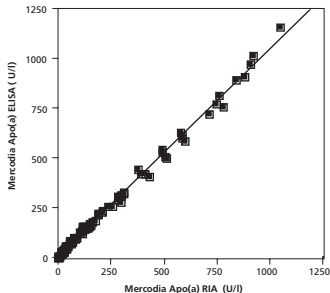
LIMITATIONS OF THE PROCEDURE

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.

COMPARISON WITH MERCODIA APO(a) RIA

Comparison studies between Merckodia Apo(a) ELISA and Merckodia 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 Merckodia Apo(a) RIA can be used for Merckodia Apo(a) ELISA as well.



EXPECTED VALUES

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

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

- A Normals1, n=171, Sweden (Caucasian)
- B Normals1, 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 Apolipoprotein (a) levels.

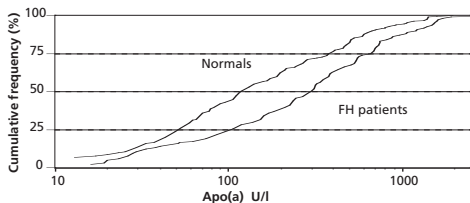
No significant difference in Apolipoprotein(a) levels was found between the group of normals from Sweden and the group of normals from Canada.

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

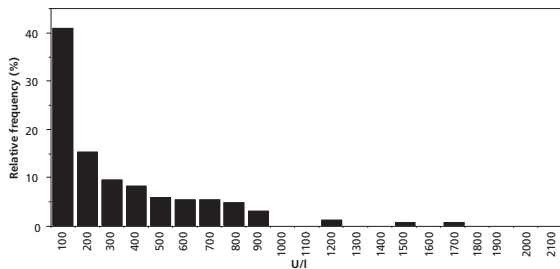
The following Apolipoprotein(a) concentrations for median, 75th, 85th and 95th percentiles were obtained for the different groups.

	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

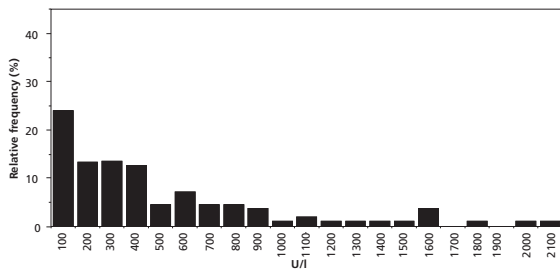
Apo(a) distribution in normals and FH patients (Canada)



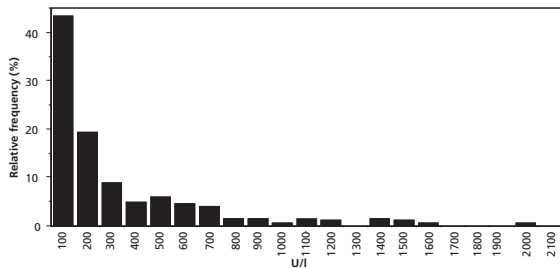
Distribution of normals (Canada)



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 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 analysed in 4-replicates on nine different occasions.

Sample	Obtained value U/l	Coefficient of variation %		
		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 analysed in 5-replicates on five different occasions.

Sample	Obtained value U/l	Coefficient of variation %		
		within assay	between assay	total assay
4	103	3.1	4.2	5.2
5	251	3.6	3.7	5.2
6	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 crossreaction.

CALIBRATION

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

The concentration of Apolipoprotein(a) is expressed in Units/l.

It is not possible to express the concentration of Apo(a) in mass units as there are at least six different isoforms described with molecular weights varying from approximately 300 kD to 900 kD (1,2,15,16). Thus each patient sample will contain different proportions of the different isoforms.

Therefore no exact conversion factor can be given between Units of Apo(a) and milligrams of Apo(a).

1 Unit of Apo(a) is approximately equal to 0.7 mg Lp(a) protein.

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 PROTOCOL SHEET/ZUSAMMENFASSUNG DES PROTOKOLLBLATTES/
FEUILLE DE PROTOCOLE RESUMEE/HOJA DE RESUMEN DEL
PROTOCOLO/PROTOCOLLO DI
SINTESI/OVERSIGTSPROTOKOLARK/SAMMANFATTNINGSPROTOKOLL
Mercodia Apo(a) ELISA**

Add Calibrators, pretreated Controls and pretreated samples	25 µl	Add Substrate TMB	200 µl
Calibrators, vorbereitete Controls und vorbereitete Proben beigegeben	25 µl	Substrate TMB beigegeben	
Ajout de Calibrators, de Controls et d'échantillons prétraités	25 µl	Ajout de Substrat (TMB)	
Añadir Calibrators, Controls pretratados y muestras pretratadas	25 µl	Añadir Substrate TMB	
Aggiungere Calibrators, Controls pre-trattati e campioni pre-trattati	25 µl	Aggiungere Substrate TMB	
Tilsæt Calibrators, forbehandlede Controls og forbehandlede prøver	25 µl	Tilsæt Substrate-TMB	
Tillsätt Calibrators, förbehandlade Controls och förbehandlade prover	25 µl	Tillsätt Substrate TMB	
Add Enzyme Conjugate	50 µl	Incubate	15 minutes
Enzyme Conjugate beifügen		Inkubieren	15 Minuten
Ajout d'Enzyme Conjugate		Incubation	15 minutes
Añadir Enzyme Conjugate		Incubar	15 min
Aggiungere Enzyme Conjugate		Incubazione	15 minuti
Tilsæt Enzyme Conjugate		Inkuber	15 min
Tillsätt Enzyme Conjugate		Inkubera	15 minuter
Incubate	1 hour at 18–25°C on a shaker	Add Stop Solution	50 µl Shake for 15 sec to ensure mixing
Inkubieren	1 Stunde auf einem Schüttler bei 18–28°C	Stop Solution beifügen	50 µl Sicherstellen von Durchmischung 15 Sek. schütteln
Incubation	1 heure à 18–25°C sur un agitateur secoueur de plaques	Ajout de Stop Solution	50 µl Secouer pendant 15 secondes pour bien mélanger
Incubar	1 hora a 18–25 °C en un agitador de placas	Añadir Stop Solution	50 µl Agitar durante 15 segundos para asegurar el mezclado
Incubazione	1 ora a 18–25° C in una piastra shaker	Aggiungere Stop Solution	50 µl Scuotere per 15 secondi per assi curarsi che sia tutto mescolato
Inkuber	1 time ved 18–25°C på et rystebord	Tilsæt Stop Solution	50 µl Ryst i 15 sekunder for sikre blanding
Inkubera	1 timme vid 18–25°C på en plattskak	Tillsätt Stop Solution	50 µl Skaka i 15 sekunder för att se till att lösningen blandas
Wash	6 times	Measure A ₄₅₀	
Waschen	6 mal	Messung A ₄₅₀	
Rinçage	6 rinçages	Mesure de A ₄₅₀	
Lavar	6 veces	Medir A ₄₅₀	
Lavare	6 volte	Misura A ₄₅₀	
Skyl	6 gange	Aflæs A ₄₅₀	
Tvätta	6 gånger	Mät vid A ₄₅₀	