Trinity Biotech

Macra® Lp(a)
Enzyme Linked Immunosorbant Assay

REF 2339100 96 Tests

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
Dla innych języków

Para outras línguas
Για τις άλλες λώσσες
För andra språk
For andre språk

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INTENDED USE

The Macra® Lp(a) Enzyme Linked Immunosorbant Assay (ELISA) kit, is an in vitro diagnostic device for the quantitative measurement of Lp(a), in human serum or plasma, for the assessment of risk for coronary heart disease (CHD) in specific populations, along with other risk factors. **For *In vitro* Diagnostic Use.**

SUMMARY AND EXPLANATION OF THE TEST

Lipoprotein (a) [Lp(a)] is a spherical lipid particle found predominantly in the 1.006 to 1.021 g/mL density range. Similar to low-density lipoprotein (LDL) in core lipid composition and having B-100 as a surface apolipoprotein (apo B), Lp(a) differs from LDL by containing an additional glycoprotein, apolipoprotein(a) [apo(a)]. The apo(a) portion of Lp(a) is heterogeneous[1-2] resulting from a variation in the number of protein domains known as kringles. One of these domains is known to repeat 12 to 52 times forming as many as 40 different isoform sizes and weights.[3]

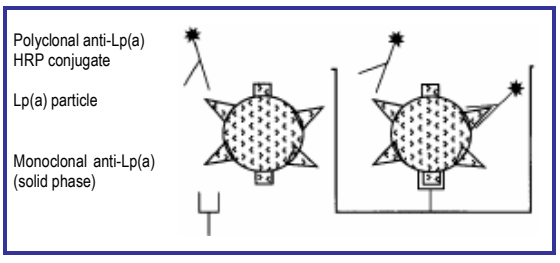
Numerous studies beginning in the 1970s have reported an association of Lp(a) with coronary heart disease (CHD).[4-8] Since then, a considerable amount of literature has been generated further documenting an association of elevated Lp(a) levels with increased risk of coronary artery disease (CAD), and premature CAD in Caucasian males.[9] Both familial studies and studies in different ethnic populations have shown that Lp(a) levels are genetically determined.[9-12] Families with a history of CAD frequently have higher than expected levels of Lp(a). The Lp(a) levels in different ethnic populations can vary widely. Africans or peoples of African decent generally have Lp(a) levels higher than Caucasians and Asians while Native Americans generally have levels lower than Caucasians. This variability of Lp(a) levels by ethnic population requires careful interpretation of results based on a knowledge of the patient and other cardiac risk factors which may be present.[13]

Appendix I provides more detailed information about the association of lipoprotein(a) levels with coronary artery disease and the expected levels in different populations.

PRINCIPLE OF THE TEST

The Macra® Lp(a) Test Kit is based on the Enzyme Linked Immunosorbant Assay (ELISA) principle. The test is a sandwich assay which utilizes both a monoclonal antibody and polyclonal antibodies which specifically bind to the apolipoprotein(a) moiety of Lp(a). The monoclonal antibody is coated in the wells of a microtiter plate (Figure 1) and used to "capture" Lp(a) from the sample during a one-hour incubation at room temperature. After washing the plate wells, a polyclonal anti-Lp(a) horseradish peroxidase (HRP) conjugate is added to bind to other sites on the Lp(a) molecule to form a sandwich. After 20 minutes the plate is washed a second time, and the Lp(a)/antibody sandwich on the plate wells is then reacted with a substrate for HRP (hydrogen peroxide) and a chromogen (o-phenylenediamine) producing a colored solution. After 20 minutes the reaction is stopped with sulfuric acid and the color developed is directly proportional to the concentration of Lp(a) in the sample. The concentration of Lp(a) mass (mg/dL) is quantitatively determined by comparison of the absorbance of the sample with a standard curve prepared with known concentrations of Lp(a).

Figure 1: Typical Well



REAGENTS SUPPLIED (SUFFICIENT FOR 96 WELLS)

All materials supplied are for in vitro diagnostic procedures. Upon receipt all reagents should be stored refrigerated (2-8ºC). The expiration dates of the components are given on the vial labels. Do not mix individual reagents from one kit lot with a different kit lot. All kit components should be used as specified,

1. Lp(a) TEST WELL STRIPS**:** 12 strips (1 x 8 wells) in a frame. Mouse anti-Lp(a) (monoclonal) immobilized on microwell strip. Keep unused strips tightly sealed in the foil pouch with the desiccant pack during storage.
2. Lp(a) STANDARDS: 1 x 2 mL each of six (6) levels. Supplied ready to use. Contains Lp(a) in human plasma in a buffered solution with protein stabilizers and ProClin®. See the "Precautions" section for further information. Bromphenol blue added for color.
3. Lp(a) CONTROLS: 1 x 2 mL each of two (2) levels. Supplied ready to use. Contains Lp(a) in human plasma in a buffered solution with protein stabilizers and Proclin. See the "Precautions" section for further information. A range for each control level is provided on the Control Data Sheet supplied with each kit. Bromphenol blue added for color.
4. Lp(a) SAMPLE DILUENT: 2 x 45 mL Buffered solution containing bromphenol blue for color, protein stabilizers and Proclin.
5. WASH BUFFER CONCENTRATE (20X): 1 x 50 mL Buffered solution containing ProClin®.
6. ANTI-Lp(a)-HRP CONJUGATE: 1 x 11 mL HRP conjugated to anti-Lp(a) (goat, polyclonal) in a buffered solution with protein stabilizers and ProClin®. Bromphenol blue added for color.
7. OPD REAGENT: 1 x 5 tablets Each tablet contains o-phenylenediamine dihydrochloride and excipients. Avoid contact with skin.
8. COLORIMETRIC REAGENT: 1 x 50 mL Buffered solution with hydrogen peroxide.
9. STOP REAGENT: 1 x 10 mL 2N Sulfuric acid. Avoid contact with eyes and skin.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Pipets: -10 µL, 100 µL
  - 50-100 µL multi-channel pipet
  - 2.0 mL, 5.0 mL, 50 Ml
- Mixer: Vortex mixer or equivalent.
- Rotator: Capable of maintaining 120 ± 5 rpm.

NOTE: Rotators/shakers vary in orbit diameter; the recommended speed applies to a rotator with a 0.75 " orbit. Rotators with different orbit diameters will need to have their speed adjusted accordingly.

- Plate Washer: Capable of washing an eight (8) well strip or 96 well plate. Adjust the volume to dispense 300 µL, per well.
- Plate Reader: A suitable microplate spectrophotometer that can measure absorbance at 492 nm (490 nm acceptable). Refer to the manufacturer's operations manual for proper installation, operation, maintenance and hazards.
- Miscellaneous
  - Glass or borosilicate test tubes
  - Pipet tips
  - Reagent reservoirs
  - Infectious waste container
  - Gloves
  - Plastic Forceps
  - One Liter graduated cylinder

PRECAUTIONS

1. Read the entire package insert before using any of the materials.
2. Samples received for analysis, as well as the kit standards and controls contain human plasma or serum and must be handled as if capable of transmitting infectious diseases such as hepatitis B and C virus or HIV type I and 2. *See the HHS publication* (CDC) 84-8395 Biosafety in Microbiological and Biomedical Laboratories.
3. Use disposable gloves when handling all specimens.
4. OPD tablets should not be allowed to come in contact with any metal surface. Use plastic forceps to handle OPD tablets.
5. The accuracy and precision of results are generally enhanced by the use of properly calibrated pipets.
6. Do not pipet by mouth.
7. Do not let wells dry out after washing.
8. Pipet all reagents into the bottom of the microwell.
9. Change pipet tips between each standard, control, reagent and sample.
10. Do not use reagents that have expired.
11. Do not mix reagents from different kit lot numbers.
12. The use of polystyrene tubes for sample storage or dilution has not been investigated for this procedure. Therefore, DO NOT USE POLYSTYRENE TUBES with this assay.
13. Assay runs should use at least two reliable controls to check the performance of the kit.
14. It is recommended that in-house plasma or serum controls be prepared and ranges established and used as additional quality control.
15. All standards, controls and samples should be run in duplicate.
16. Remove strips from the frame that will not be used in given assay; store appropriately (see Reagents Supplied section). Label all strips in use. If necessary, replace the removed strips with old "used" strips, if required for plate washing.
17. Better precision will be achieved by removing all residual liquid from plate by inverting and forcefully striking the plate on an absorbent pad after washing. **NOTE***: Be sure the well strips are labeled in case they dislodge from the frame.*
18. Handle the plate by the frame to avoid contact with well bottoms, since this may affect your results.
19. Wash your hands thoroughly after the completion of the test procedure.

The safety data sheet is available upon request.

WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + **P352**: IF ON SKIN: Wash with plenty of soap and water.

P333 + **P313**: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed

P264: Wash thoroughly with plenty of soap and water after handling

P270: Do not eat, drink or smoke when using this product

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P330: If swallowed, rinse mouth

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

SAMPLE COLLECTION AND STORAGE

The following guidelines, based on recommendations from the NCEP Laboratory Standardization Panel for lipid and lipoprotein testing, will minimize the effect of preanalytical factors on Lp(a) testing.

1. A subject's lipid and lipoprotein profile should only be measured when the individual is in a steady metabolic state.
2. Subjects should maintain their usual diet and weight for at least 2 weeks prior to the determination of their lipids or lipoproteins.
3. Additional measurements should be performed within 2 months and at least I week apart before making a medical decision about future action.
4. Subjects should not perform vigorous physical activity during the 24-hour period prior to testing
5. Fasting or non-fasting specimens can be used for Lp(a).
6. The subject should be seated for at least 5 minutes before specimen collection.
7. The tourniquets should not be kept on more than I minute during venipuncture.
8. All blood specimens should be considered potentially infectious and handled accordingly.
9. Either a serum or plasma sample may be used with this test. EDTA or Heparin may be used as the anticoagulants for plasma. In general, EDTA is the anticoagulant of choice for a lipid/lipoprotein panel since Heparin has been shown to artifactually lower triglyceride values.
10. Serum and plasma Lp(a) samples require special handling. Samples may be kept refrigerated (2-8'C) if analysis is to occur within 48 hours of collection. Otherwise, samples should be stored at -80'C until analysis.
11. Niacin and estrogen supplements are known to decrease Lp(a) levels.[14-21] The use of beta-blockers, other antihypertensive drugs, alcohol, and cigarettes, which are known to affect other lipids, do not influence Lp(a) levels.

Blood should be drawn using standard venipuncture techniques in either EDTA tubes or serum tubes stored on ice. For serum, the blood should clot for about 30-60 minutes at 2-8ºC before being separated. For EDTA samples, the plasma should be separated from the cells by a short refrigerated centrifugation step according to NCEP guidelines. The use of polystyrene containers for sample storage has not been investigated with this procedure and, therefore, is not recommended.

REAGENT PREPARATION

1. Allow all reagents to reach room temperature (18-25ºC) prior to use. Mix all reagents thoroughly prior to use.
2. Sample Preparations (1:201): Dilute each sample 1:201 with sample diluent by mixing 10 µL of sample with 2.0 mL of diluent in glass tubes and vortex. Change pipet tips for each new sample. DO NOT USE POLYSTYRENE TUBES for the sample dilution.
3. Wash Buffer (20X): Prepare wash buffer by diluting 50 mL of concentrate with distilled or deionized water to a final volume of I liter. Allow any crystals in the concentrate to dissolve at room temperature followed by thorough mixing before using. The diluted wash buffer may be stored at room temperature for 2 weeks or 3 months at 2-8ºC. At least 300 mL is required per plate.
4. Color Developing Solution: Prepare sufficient color developing solution fresh 20 minutes prior to use by dissolving one OPD tablet in 5 mL of colorimetric reagent for every 40 test wells. Solution should be clear to pale yellow. Do not use if dark yellow.

ASSAY PROTOCOL

1. Allow reagents to reach room temperature (18-25ºC) prior to use. Mix all reagents thoroughly before use.
2. The antibody-coated plate is packaged with a desiccant pack. Care should be taken to ensure that the desiccant pack is not damaged when the pouch is opened. Return unused Lp(a) Test Well Strips to the original pouch with the desiccant pack provided and seal pouch with tape.
3. Strip well layout: Figure 2 is provided as a guide for a typical plate layout.

Sample	Code
0 mg/dL Standard	S1
5 mg/dL Standard	S2
10 mg/dL Standard	S3
20 mg/dL Standard	S4
40 mg/dL Standard	S5
80 mg/dL Standard	S6
Control Level 1	C1
Control Level 2	C2
Unknown I	U1
Unknown 2	U2
Etc.	

Figure 2

A	S1	S1	Ul	Ul	U9	U9
B	S2	S2	U2	U2	U10	U10
C	S3	S3	U3	U3	U11	U11
D	S4	S4	U4	U4	U12	U12
E	S5	S5	U5	U5	U13	U13
F	S6	S6	U6	U6	U14	U14
G	C1	C1	U7	U7	U15	U15
H	C2	C2	U8	U8	U16	U16
	1	2	3	4	5	6

4. Dilute all serum/plasma samples 1:201 in glass tubes by adding 10 µL, of sample to 2.0 mL of Sample Diluent. Vortex briefly to mix. DO NOT USE POLYSTYRENE TUBES for the sample dilution.
5. Pipet 100 µL of the standards and controls, in duplicate, into the bottom of the test wells as shown in Figure 2

NOTE: The standards and controls are provided at a 1:201 dilution, therefore, do not dilute these reagents with sample diluent.

6. Pipet 100 µL of the diluted samples, in duplicate, into the bottom of their respective wells.
7. Incubate at a controlled room temperature (18-25ºC) for I hour ± 2 minutes on an orbital rotator at 120±5 rpm. Start timing after the addition of the last sample.
8. Washing:
   - Semi-automated/Automated Washing. Aspirate the plate and wash two times with a microplate washer. Turn the plate 180 degrees and wash two additional times Invert the plate and firmly tap on absorbent pad to remove any excess wash solution.
   - Manual Washing. Alternatively, using an eight or twelve-channel manifold, aspirate and wash each well four times. Invert the plate and firmly tap on absorbent pad to remove any excess wash solution.

NOTE. Precision will be enhanced by removing as much residual liquid as possible. Labeling the test strips will define strip position in case the strip becomes dislodged from the frame.

9. Using a multi-channel pipet add 100 µL of Anti-Lp(a)-HRP Conjugate into the bottom of each well.
10. Incubate at a controlled room temperature (18-25ºC) for 20 ± I minutes on an orbital rotator at 120 ± 5 rpm.
11. When the conjugate incubation is started, prepare the Color Developing Solution by adding one OPD Tablet per 5.0 mL of Colorimetric Reagent. This is enough material for five strips. Allow the tablet(s) to completely dissolve then vortex.
12. Repeat Washing Step 8.
13. Using a multi-channel pipet with clean tips, pipet 100 µL of Color Developing Solution into each well and incubate at a controlled room temperature (18-25ºC) for 20 ± I minutes on an orbital rotator at 120 ± 5 rpm. It is advisable to add the Color Development Solution at timed intervals and to add the Stop Reagent at the same intervals after the color development step.

NOTE: If the absorbance values of the Standards are higher than the linear range of the available instrumentation, reducing the timing of the colorimetric development step from twenty (20) minutes to a minimum of eight (8) minutes will not otherwise affect assay performance. If the colorimetric development step is reduced, particular attention should be paid to the timing of stop reagent addition.

14. Pipet 50 µL of Stop Reagent into each test well using a multi-channel pipet.
15. Blank the plate reader on a 0 mg/dL Standard well. Read the plate at 492 nm (490 nm acceptable) within 15 minutes of adding the Stop Reagent.

QUALITY CONTROL

1. Do not use reagents past their expiration date.
2. Do not interchange reagent from one kit lot with another kit lot.
3. Always run at least two reliable controls to verify the performance of the kit.
4. We recommend that in-house control plasma or serum controls be prepared and ranges established and used for additional quality control.
5. Control data for in house control samples should be plotted on a Levey-Jennings type chart to evaluate result trends. For additional information see:

   Levey S, Jennings EK The rise of control charts in the clinical laboratory. *Am J Clin Pathol* 20: 1059; 1950.
6. All values for kit supplied controls should be within their published range for assay run validation, ensuring an accurate, reportable patient sample result.
7. Numerous references are available on establishing assay quality control programs. For guidance or additional information, see:

   Westgard JO, Groth T, et al. Principles for developing improved quality control procedures. *Clin Lab Invest Suppl* 172: 19-41; 1984.
   Dudley RA, Edwards P, et al. Guidelines for immuno-assay data processing. *Clin Cbem* 31(8): 1264-1271; 1985.
   Westgard 10, Stein B, Automated selection of statistical quality-control procedures to assure meeting clinical or analytical quality requirements. *Clin Chem* 43(2): 400-403; 1997.

RESULTS

NOTE: ALL DATA PRESENTED ARE FOR DESCRIPTIVE PURPOSES ONLY. THESE DATA SHOULD NEVER BE SUBSTITUTED FOR VALUES OBTAINED USING THE ASSAY KIT.

1. Average the absorbance readings for duplicates of the standards, controls and unknowns.
2. Plot a curve of mean absorbance versus mg/dL Lp(a) for each standard (see Figure 3 and Table 1). Either a point-to-point, 4 parameter or log-log curve fitting transformation may be used.
3. Derive unknown and control values from the standard curve as illustrated in Figure 3 and Table 1. Ensure the control results are within accepted quality control criteria prior to reporting sample results.

Figure 3: Typical Standard Curve

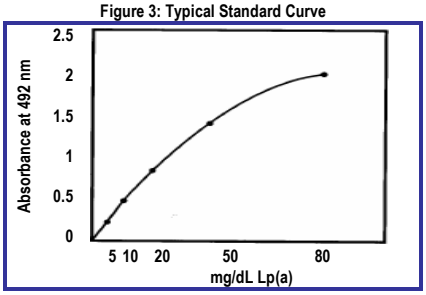


Table 1
Sample Data

Sample	Code	Abs	Mean Abs	Lp(a) Value mg/dL	% O.D. CV
0 mg/dL STD	S1	0.000	0.000	-	-
			-0.001		
5 mg/dl STD	S2	0.257	0.257	-	0.0
			0.257		
10 mg/dL STD	S3	0.468	0.467	-	0.4
			0.465		
20 mg/dL STD	S4	0.835	0.832	-	0.4
			0.830		
40 mg/dL STD	S5	1.396	1.391	-	0.6
			1.385		
80 mg/dL STD	S6	2.014	2.000	-	1.1
			1.985		
Control Level 1		0.746	0.733	16.7	2.5
			0.720		
Control Level 2		1.343	1.323	36.8	2.1
			1.303		
Unknown 1		0.157	0.156	3.0	1.0
			0.155		
Unknown 2		1.250	1.242	34.7	0.6
			1.233		

Etc.

NOTE: A correction for the specimen dilution of 1:201 is not necessary since the standards are provided in a prediluted form.

LIMITATIONS OF THE PROCEDURE

1. Extrapolating values from the curve that are less than 5 mg/dL or greater than 80 mg/dL is not recommended. Serum or plasma samples with Lp(a) concentrations greater than 80 mg/dL or less than 5 mg/dL should be reported as such.
2. If a quantitative value is desired for a sample greater than 80 mg/dL, the sample should be further diluted into the assay range with Sample Diluent The value obtained should be multiplied by this dilution factor to obtain the mg Lp(a)/dL value.
3. Samples should not be preserved with Sodium Azide since this can affect the color development process.
4. Potential Interferences - Samples which contain the following substances at concentrations up to those listed do not interfere with the assay's ability to quantitate Lp(a).
   a. Triglyceride - 2500 mg/dL
   b. Hemoglobin - 5 g/dL
   c. Bilirubin - 2.5 mg/dL
5. Either a serum or plasma sample may be used with this test. EDTA or Heparin may be used as the anticoagulants for plasma. In general, EDTA is the anticoagulant of choice for a lipid/ lipoprotein panel since Heparin has been shown to artifactually lower triglyceride values.
6. Serum and plasma Lp(a) samples require special handling. Samples may be kept refrigerated (2-8ºC) if analysis is to occur within 48 hours of collection. Otherwise, samples should be stored at -80ºC until analysis.
7. Niacin and estrogen supplements are known to decrease Lp(a) levels.[14-21] Treatment regime should not be initiated based upon a single Lp(a) value.

EXPECTED VALUES

Lipoprotein (a) levels are influenced by genetic factors and vary with ethnic population. The following expected levels for healthy subjects are for reference only. It is recommended that each laboratory establish its own reference range with consideration for the assay method being used. For assistance in determining reference intervals that meet the minimum requirements for reliability and usefulness, refer to NCCLS document "C28-A How to Define and Determine Reference Intervals in the Clinical Laboratory, Approved Guideline 1995".

Population	Expected Lp(a) Levels in mg/dL (Mean ± S.D.)	References (Representative Sample Size)	
Caucasians / Asians	14 ± 17	9, 22, 23	(2678)
Mexican Americans	11 ± 1.1	24, 25	(316)
Africans & Descendants	28 ± 22	10, 22, 26	(4165)
Native Americans & Descendants	7 ± 14	24, 25, 27	(4500)

The available information suggests that the use of a Lp(a) mass level of 30 mg/dL as a conservative risk differentiation level for Caucasian populations, when used in conjunction with other documented cardiac risk factors, provides a useful tool for determining risk of premature CHD especially in Caucasian men.[28,29] However, considering the nature of lipid and lipoprotein CHD risk indicators, patients with Lp(a) levels above 20 mg/dL may warrant close monitoring especially if other risk factors are present.

Although an Lp(a) level above 30 mg/dL as determined by the Macra® Lp(a) assay, increases the likelihood of an individual developing atherosclerosis or CHD, not all individuals with elevated Lp(a) will develop significant disease. In some individuals, an Lp(a) value below 30 mg/dL does not rule out significant atherosclerosis or CHD. The assessment of such disease should be made with consideration of the patient's history, ethnicity, clinical information, and other clinical laboratory tests.

Additional data is being collected to help establish expected ranges for other populations.

SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY OF DILUTION

Three samples were diluted with Sample Diluent and assayed for Lp(a) concentration. The percent recovery value was calculated by dividing the observed value by the expected value and multiplying by 100 (**NOTE:** Each sample was previously diluted 1:201*)*.

Dilution	Sample	Expected (mg/dL)	Observed (mg/dL)	% Recovery
1:1	1	68.1	68.1	100
3:4		51.1	49.2	96
1:2		34.1	36.8	108
1:4		17.0	17.9	105
1:8		8.5	8.5	100
1:1	2	40.1	40.1	100
3:4		30.0	31.8	106
1:2		20.0	21.8	109
1:4		10.0	10.7	107
1:8		5.0	4.8	96
1:1	3	29.8	29.8	100
3:4		22.4	21.8	97
1:2		14.9	15.1	101
1:4		7.5	7.8	104
1:8		3.7*	3.1	4

*Outside recommended assay range.

RECOVERY

Known quantities of Lp(a) were added to two diluted specimens. The recovery was calculated by dividing observed value by the expected value and multiplying by 100.

Sample	Lp(a) Value (mg/dL)	Spiked Amount (mg/dL)	Expected (mg/dL)	Observed (mg/dL)	% Recovery
1	12.3	0.0	12.3	12.3	100
		5.0	17.3	16.1	93
		10.0	22.3	21.9	98
		20.0	32.3	26.8	83
		40.0	52.3	42.0	80
2	31.3	0.0	31.3	31.3	100
		5.0	36.3	33.5	92
		10.0	41.3	38.3	93
		20.0	51.3	46.7	91
		40.0	71.3	64.4	90

PRECISION

Intra-assay Variation

Data for the within run precision were obtained from five human plasma samples by running each sample in four replicates.

Sample	Mean (mg/dL)	S.D.	% C.V.
1	7.7	0.8	1.4
2	8.1	0.5	6.4
3	14.5	0.6	4.0
4	28.0	2.0	7.0
5	65.7	2.0	6.3

Inter-assay Variation

Data for the " between " run precision were obtained by running four human plasma samples in duplicate in eight assays.

Sample	Mean (mg/dL)	S.D.	% C.V.
1	10.3	0.8	7.8
2	18.3	1.1	6.0
3	37.8	3.2	8.5
4	67.8	8.6	12.7

SPECIFICITY

The following substances were added to human plasma samples containing varying levels of Lp(a). No detectable cross-reactivity was observed up to the levels indicated.

Compound	Concentration	Cross-Reactivity
Plasminogen	200 mg/dL	None
LDL	166 mg/dL	None
HDL	400 mg/dL	None
VLDL	200 mg/dL	None

MINIMUM DETECTION LEVEL

The minimum detection level of the Lp(a) assay was obtained by running the standard curve five times, calculating the mean and standard deviations for the calibrators, adding two standard deviations to the mean absorbance for the 0 mg/dL Standard and interpolating the Lp(a) value. The Lp(a) value obtained was consistently less than or equal to 0.8 mg/dL.

CORRELATION WITH OTHER METHODS: SEE APPENDIX II

Appendix I - Background of lipoprotein (a), its association with risk of coronary heart disease and variations in different populations

Lipoprotein (a) [Lp(a)] is a spherical lipid particle found, upon ultracentrifugation, predominantly in the 1.006 to 1.021 g/mL density range. Similar to low-density lipoprotein (LABEL) in core lipid composition and having B-100 as a surface apolipoprotein (apo B), Lp(a) differs from LDL by containing an additional glycoprotein, apolipoprotein(a) [apo(a)]. Apo(a) is covalently bound to apolipoprotein B-100 (apo B) through a disulfide bond. Studies have shown that the apo(a) portion of Lp(a) is heterogeneous.[1-2] This heterogeneity results from a variation in the number of protein domains known as kringles. One of these domains, kringle 4, is known to repeat, forming many

different isoform sizes and weights. As many as 40 different isoforms have been reported, having from 12 to 52 repeats of kringle 4.[3]

Both the cDNA sequence[30] and the immunochemical structure of apo(a) have been shown to be strikingly similar to the kringle 4 and 5 domains of plasminogen. Because of this similarity, Lp(a) inhibits fibrinolysis by competition with plasminogen for fibrin.[31,32] In addition, Lp(a) deposits in atherosclerotic plaque promoting clogging of arteries.[33,34] Krempler[35] have shown that apo(a) is synthesized independently of other lipoproteins and then attaches itself to apo B containing particles in plasma. Albers[36] demonstrated however, that plasma apo B levels do not correlate with Lp(a) levels. Lp(a) was first reported by Kate Berg of the University of Oslo in 1963.[11] Numerous studies in the 1970s reported an association of Lp(a) with coronary heart Disease (CHD).[4-8] Since then, a considerable amount of literature has been generated further documenting an association of elevated Lp(a) levels with increased risk of coronary artery disease (CAD).

The association of elevated levels of Lp(a) with risk of myocardial infarction (MI) has been documented and has substantiated Lp(a) to be an independent risk factor for M1.[37-39] The relative risk for MI in individuals with Lp(a) levels greater than 30 mg/dL was reported to be 1.85 times higher than in individuals with Lp(a) levels below 30 mg/dL.[33] As a result, 30 mg/dL has frequently been cited as the level where one is considered at higher risk. Numerous studies have shown an association of elevated levels of Lp(a) with stroke or cerebrovascular disease and peripheral vascular disease.[40-50] These studies included individuals of Caucasian, Japanese and Chinese origin. Elevated Lp(a) was concluded to be an independent risk factor for CAD in Caucasian patients with familial hypercholesterolemia and familial lipoprotein disorders.[9,28,51] Clinical studies have shown elevated levels of Lp(a) to be associated with stenosis[52,53] and restenosis after percutaneous transluminal coronary angioplasty (PTCA).[54,55] Studies show that Lp(a) levels are almost entirely determined by genetic factors and elevated levels of Lp(a) arc commonly seen in patients and families with premature CHD. Familial studies conducted in the United States and in Europe have shown Lp(a) is inherited through a major monogenic pattern influenced by other genes.[56] Thus, Lp(a) may be an important initiator and promoter of, as well as an early marker for, the atherosclerotic process. Numerous retrospective case-control studies have been conducted and show a link between high Lp(a) levels and CHD.[37,51,57-70]

Standard lipid-lowering regimens have been unsuccessful in lowering Lp(a) levels. Lp(a) levels appear to be insensitive to diet, HMG-CoA reductase inhibitors (statins), bile acid sequestrants and probucol. Treatment with niacin at 3 grams per day in men and women, [14,15] or estrogen or an estrogen/progestin combination in postmenopausal (primarily Caucasian) women significantly decrease Lp(a) levels.[16-21] Both these treatment modalities have been associated with reduction in CHD morbidity and mortality. Therefore, Lp(a) should be monitored in patients with CHD, patients with a strong family history of CHD and patients who are candidates for drug therapy for LDL-cholesterol (LDL-c) lowering as per Adult Treatment Panel I (ATPI) protocol of the National Cholesterol Education Program (NCEP).[71]

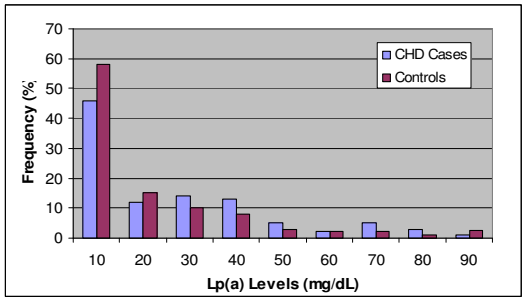
Lp(a) levels are genetically determined and the normal range of Lp(a) in humans varies by ethnic population.[9-12] The Framingham Offspring study[23] of 2678 Caucasian men and women (ages 20 -70 + mean 48) who were free of symptoms of atherosclerotic disease show a highly skewed Lp(a) distribution (mean ca. 14.5 mg/dL; median ca. 7.9 mg/dL) with 56% of subjects having values between 0 and 10 mg/dL. Appendix I Table I shows the percentiles for plasma levels of Lp(a) for this study. The mean ± SD values for men of 14 ± 17 and for women of 15 ± 17 are very similar and change very little with age. These findings are consistent with other studies.[9,22] Studies[10,22,26] have shown that Africans or peoples of African decent have normal levels of Lp(a) two to three times higher than Caucasians with distributions less skewed (mean ca. 28.5 mg/dL; medium ca. 22.9 mg/dL) (Table 1). Native American populations are reported[27] to have Lp(a) levels about half those of Caucasian populations and Mexican Americans[24] have Lp(a) levels significantly lower (ca. 30%) than Caucasian populations and certain sub-populations of Mexican Americans have Lp(a) levels half those of Caucasians. Asian populations generally have mean Lp(a) values and distributions similar to Caucasian populations.[12,72,73]

Appendix I Table 1
Percentile Distribution of Lp(a) in Two Population Studies using the Macra® Lp(a) Test Kit.
Values are in mg/dL of Lp(a)

	CARDIA Study (23-35 years old)				Framingham Study	
	Black (mg/dL)		White (mg/dL)		White (mg/dL)	
Percentile	Men	Women	Men	Women	Men	Women
95	67.9	72.9	52.7	51.5	49.6	52.9
90	58.1	62.1	39.1	39.9	38.0	37.5
75	37.9	42.8	19.8	20.2	21.2	22.7
50	21.5	23.9	6.1	6.4	7.6	8.2
25	9.8	12.1	2.2	2.8	2.6	3.0
10	4.4	5.5	1.0	1.3	1.0	1.1
5	2.0	2.9	0.6	0.9	0.6	0.7
N	861	1128	1011	1125	1284	1394
Mean ± SD	27 ± 22	30 ± 24	14 ± 17	14 ± 17	14 ± 17	15 ±17

Several studies have shown that the onset of CHD in Caucasian men, as determined by both myocardial infarction[29,63] and angiography,[28,57] begins to be evident in the 70th to 80th percentile range of Lp(a) levels in subjects. Appendix I Figure I provides data from a case-control study[28] of 321 men with angiographically documented CAD (determined during elective angiography) and 901 control subjects from the Framingham Offspring Study. The data show that the frequency of CHD cases by angiography begins to exceed that of controls at Lp(a) levels, determined by Macra®, between 20 and 30 mg/dL and becomes more significant above 30 mg/dL. In the total Framingham Offspring Study of 1210 CHD-free men the Lp(a) range between the 75th and 80th percentile was 21-27 mg/dL and between the 80th and 90th percentiles was 27-38 mg1dL.

Appendix I Figure 1
The Framingham Offspring Study



Additional information from this study compares (Appendix I Table 2) other lipid and lipoprotein cardiac risk factors for this population with Lp(a). The study28 also included adjusting the data to account for differences in diet and use of beta-blockers.

Appendix I Table 2
Lipoprotein and Apolipoprotein Levels in Control Subjects and Patients with Coronary Artery Disease (Mean +/- SD)

Risk Factor	Control Group	Coronary Artery Disease (n=321)			
	(n=901)	Overall	P1	After Adj*	P2
T Chol	214 ± 36	211 ± 49	0.343	224 ± 53	<0.001
Tg	141 ± 104	189 ± 96	<0.001	189 ± 95	<0.001
VLDL	28 ± 21	38 ± 19	<0.001	38 19	<0.001
LDL	138 ± 33	141 ± 46	0.853	156 51	<0.001
HDL	45 ± 12	35 ± 10	<0.001	36+11	<0.001
Apo B	108 ± 33	123 ± 33	<0.001	131 ± 37	<0.001
Apo A- 1	136 ± 32	111 ± 25	<0.001	114 ± 26	<0.001
Lp(a)	14.9 ± 17.5	19.9 ± 21.5	<0.002	19.9 ± 19	<0.003

* Adjusted for diet and beta-blockers.

The data from these several study[9,28,51] indicate that Caucasians with Lp(a) levels, as measured by Macra®, which are at or above the 80th percentile (approx. 27 mg/dL) of their respective populations are more likely to have premature CHD. The indications are significant for premature CHD in men. The studies indicate that risk of CHD as measured by MI begins to be evident in men with hypercholesterolemia[29] at Lp(a) levels below 30 mg/dL. Also when CHD is determined by angiography, evidence of CHD appears in the range of 20-30 mg/dL.[28] For women, the low incidence of CHD cases to date in the on-going Framingham Offspring Study do not provide a significant indication although the association of CHD in women with elevated Lp(a) levels could be established as further information becomes available. To date insufficient population study data is available for populations other than Caucasians to establish an association between Lp(a) levels and risk of CHD.

Appendix II - Correlation with other methods

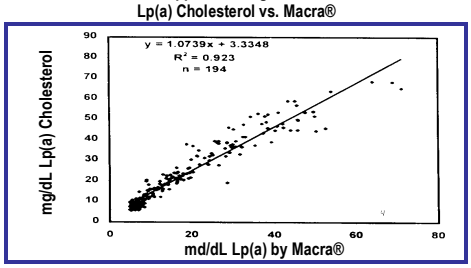
Ultracentrifugation-Paper electrophoresis[63]

The Macra® Lp(a) kit was correlated to the ultracentrifugation paper electrophoresis method used to qualitatively determine the presence of the sinking pre-beta band { Lp(a) }. Two thousand eight hundred and fourteen samples (2814) were run using both electrophoresis and the Macra® method. The electrophoresis methodology involved ultracentrifuging the sample at a density of 1.006, collecting the sinking fraction and performing paper electrophoresis on the sinking fraction. A sole individual blinded to the Macra® results was asked to score the sinking pre-beta band as none, borderline or definite. Using Lp(a) > 30 mg/dL as a cutoff and "definite" sinking pre-beta band grouping as the gold standard to calculate sensitivity and specificity, we have the above results.

Lp(a) Cholesterol Method[62]

The Macra® Lp(a) kit was compared to an Lp(a) cholesterol method." This method uses lectin affinity to isolate the Lp(a) and then measures the cholesterol from the isolated fraction. Two hundred thirty eight (238) plasma samples within the Macra® assay range (5-80 mg/dL) were analzyed comparing Lp(a) cholesterol mass (mg/dL) to total Lp(a) lipoprotein total particle mass (mg/dL). The linear regression analysis is given in Appendix II Figure 2. Note the slope of the line is 0.22. This value is thought to be due to the % of Lp(a) cholesterol mass (approximately 25%) relative to the total Lp(a) particle mass.

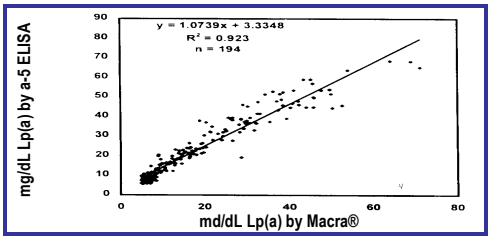
Appendix II Figure 2:
Lp(a) Cholesterol vs. Macra®



NWLRL Monoclonal antibody a-5 ELISA[75]

The Macra® Lp(a) kit was correlated to the published method from the Northwest Lipid Research Laboratories (NWLRL) in Seattle, Washington using the a-5 monoclonal antibody. NWLRL compared the Macra® and a-5 ELISA results for one hundred ninety four (194) plasma samples (CARDIA population subset) within the Macra® assay range. The linear regression analysis is given in Appendix II Figure 3.

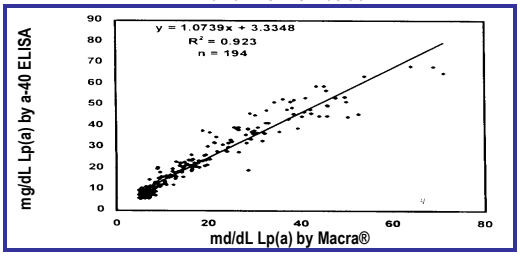
Appendix II Figure 3:
NWLRL a-5 ELISA vs. Macra®



NWLRL Monoclonal antibody a-40 ELISA[75]

The Macra® Lp(a) kit was correlated to the published method from the Northwest Lipid Research Laboratories (NWLRL) in Seattle, Washington using the a-40 monoclonal antibody. NWLRL compared the Macra® and a-40 ELISA results for one hundred ninety one (191) plasma samples (CARDIA population subset) within the Macra® assay range. The linear regression analysis is given in Appendix II Figure 4.

Appendix II Figure 4:
NWLRL a-40 ELISA vs. Macra®



REFERENCES

1. Koschinsky ML, Beisiegel U, et al. Apolipoprotein(a) size heterogeneity is related to variable number of repeat sequences in it mRNA. *Biochemistry* 29: 640-644; 1990.
2. Utermann G. The Mysteries of Lipoprotein(a) *Science* 246: 904-910; 1989.
3. Marcovina SM, ZhangeZH, et al. Identification of 34 apolipoprotein(a) isoforms: Differential expression of apolipoprotein(a) alleles between American Blacks and Whites. *Biocbemical and Biophysical Research Communications* 191/3: 1192-1196; 1993.
4. Berg K A new serum type system in man: the Lp system. *Acta Parbol Microbiol Scand* 59: 362-382; 1963.
5. Insull W, Najrni M, et al. Plasma pre-beta lipoprotein subfiractions in diagnosis of coronary artery disease. *Circulation* 45/2: 170; 1972.
6. DaWcn G, Ericson C, et al. Angina of effort and an extra pre-beta lipoprotein fraction. *Acta Med Scan Suppl* 531: 11-15; 1972.
7. Berg K, Dahlen G, Frick MH. Lp(a) lipoprotein and pre-bcta lipoprotein in patients with coronary heart disease. *Clin Genet* 6: 230-235; 1974.
8. Dahlen G, Berg K, et al. Lp(a) lipoprotein/prc -beta lipoprotein in Swedish middle-aged males and in patients with coronary heart disease. *Clin Genet* 7: 334-341; 1979.
9. Gencst J, Jenner JL, et al. Prevalence of lipoprotein(a) [Lp(a)] excess in coronary artery disease. *Am J Cardiol* 67: 1039-1045; 1991.
10. Marcovina SM, Albers JJ, Jacobs DR, et al. Lipoprotein(a) concentrations and Apolipoprotein(a) Phenotypes in Caucasians and African Americans: The CARDIA Study, *Arteriosclerosis and Tbrombosis* 13: 1037-1045; 1993.
11. Sandholzer C., Saba N., Kark JD, et al. Apo(a) isoforms predict risk for coronary heart disease: a study in six populations. *Arterioscler Thromb* 12: 1214-26; 1992.
12. Cobbaert C, Kesteloot H. Serum lipoprotein(a) levels in racially different population. *Am J Epidemiology* 136: 441-9; 1992.
13. Handbook of Lipoprotein Testing. Ed Rifai N, Warnick GR, Dominiczak MH, AACC Press Washington DC 294-297; 1997.
14. Gurakar A, Hoeg JM, et al. Levels of lipoprotein Lp(a) decline with neomycin and niacin treatment. *Atherosclerosis* 57: 293-301; 1985.
15. Kostner GM. The affection of lipoprotein(a) by lipid lowering drugs in recent aspects of diagnosis and treatment of lipoprotein disorders: impact on prevention of atherosclerotic diseases. K. Wildhom and N Naito, eds. 255-263; 1988.
16. Soma MR, Meschia M, et al. Hormonal agents used in lowering lipoprotein(a). *Chemistry and Physics of Lipids*67/68: 345-350; 1994.
17. Nabulsi AA, Folsom AR, White, et al. Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. *N Eng J Med* 328: 1069-1075; 1993.
18. Henriksson P, Angelin B, et al. Hormonal regulation of serum Lp(a) levels: opposite effects after estrogen treatment and orchidectomy in males with prostatic carcinoma. *J Clin Invest* 89: 1166-1171; 1992.
19. Soma MR, Osnago-Gadda 1, et al. The lowering of lipoprotein(a) induced by estrogen plus progesterone replacement therapy in postmenopausal women. *Arch Intern Med* 153: 1462-1468; 1993.
20. van der Mooren MJ, Demacker PN, et al. Beneficial effects on serum lipoproteins by *17* B-oestradiolhydrogesterone therapy in postmenopausal women: a prospective study. *Eur J Obstet Gynecol Reprod Biol* 47: 153-160; 1992.
21. Seed M, Crook D. Postmenopausal hormone replacement therapy, coronary heart disease and plasma lipoproteins. *Curr Opin Lipidol* 5: 48-58; 1994.
22. Howard BV, Ngoc-Anh L, et al. Concentrations of Lp(a) in Black and White Young Adults: Relations to Fisk Factors for Cardiovascular Disease. *Ann Epidemiol* 4: 341-350; 1994.
23. Jenner JL, Ordovas JM, Lamon-Fave S, et al. Effects of Age, Sex and Menopausal Status on Plasma Lipoprotein(a) Levels. *Circulation* 87:1135-1141; 1993.
24. Haffner SM, Gruber KK, Morales PA, et al. Lipoprotein(a) Concentrations in Mexican Americans and Non-Hispanic Whites: The San Antonio Heart Study. *Am J Epidemiology* 136: 1060-1068; 1992.
25. Private communication with Dr. David C. Robbins of the Medlantic Research Institute (January *15, 1998)* indicated that the Lp(a) studies in the Strong Heart Studies for Native Americans and the San Antonio Heart Study of Mexican Americans were done with Macra® Lp(a) Test Kit. A publication is in progress relating to the Lp(a) data as part of the Strong Heart Study.
26. Schreiner PJ. Chemistry and Physics of Lipids, Lipoprotein(a) as a risk factor for preclinical atherosclerotic disease in a biracial cohort: The Atherosclerosis Risk in Communities (ARIC) Study. 67/68: 405-410; 1994.
27. David C. Robbins. Interactions of Obesity, Lipids and Diabetes with Lp(a): The Strong Heart Study (SHS), Steering Committee, Washington, DC, 1994.
28. Genest J, McNamara JB, et al. Lipoprotein cholesterol, apolipoprotein A- I and B and lipoprotein(a) abnormalities in men with premature coronary artery disease. *J Am Coll Cardiol* 19: 792-802; 1992.
29. Rosengren A, Wilhelllmsen L, Eriksson E, et al. 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; 1990.
30. McLean JW, Tomlinson JE, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 330: 132-137; 1987,
31. Gonzalez-Gronow Me Edelberg JM, Pizzo SV. Further characterization of the cellular plasminogen binding site. *Biochemistry* 28: 2374-2377; 1989.
32. Smith E, Cochran S, et al. Factors influencing the accumulation in fibrous plaques of lipid derived from low density lipoprotein 11. *Atherosclerosis* 84: 173-181; 1990,
33. Kostner G, Avogaro P, et al. Lipoprotein Lp(a) and the risk for myocardial infarction. *Atherosclerosis (Netherlands)* 38: 51-61; 1981.
34. Bisiegel U, Niendorf A, et al. Lipoprotein(a) in the arterial wall. *Eur Heart J* 11 (supplement E): 174-183; 1990.
35. Krempler F, Kostner GM, et al. Lipoprotein(a) is not a metabolic product of other lipoproteins containing apolipoprotein-B. *Biochim Biophys Acta* 575: 63-70; 1979.
36. Albers JJ, Cabana VG, et al. Lp(a) lipoprotein: relationship to pre-beta lipoprotein, hyperlipoproteinemia, and apolipoprotein-B. *Metabolism* 9: 1047-1054; 1975.
37. Kark JD, Sandholzer C, et al. Plasma Lp(a), apolipoprotein(a) isoforms and acute myocardial infarction in men and women: a case control study in Jerusalem population. *Atherosclerosis 98: 139-151; 1993.*
38. Graziani MS, Zanolla L, Righetti G, et al. Lipoprotein(a) concentrations are increased in patients with myocardial infarction and angiographically normal coronary arteries. *Eur J Berlin Chem Clin Biocbem* 3 1: 135-137; 1993.
39. Hoefler G, Harnoncourt F, et al. Lipoprotein Lp(a): A risk factor for myocardial infarction. *Atherosclerosis* 18: 398; 1988.
40. Zenker G, Koltringer P, et al. Lipoprotein Lp(a) as a strong indicator for cerebrovascular disease. *Stroke* 17:942;1986.
41. Koltringcr P, Jurgens G. A dominant role of Lipoprotein(a) in the investigation and evaluation of parameters indicating the development of cervical atherosclerosis. *Atherosclerosis* 58: 187-198; 1985.
42. Murai A, Miyahara T, et al. Lp(a) lipoprotein as a risk factor for coronary heart disease and cerebral infarction. *Atherosclerosis* 59: 199-204; 1986.
43. Woo J, Lau E, et al. Hypertension, lipoprotein(a) and apolipoprotein Al as risk factors for stroke in the Chinese. *Stroke* 22: 203-208; 1991.
44. Jurgens G, Koltringer P. Lipoprotein(a) in ischemic cerebrovascular disease: A new approach to the assessment of risk for stroke. *Neurology* 37: 513-515; 1987.
45. Woo J, Lam CWK, et al. Acute and long-term changes in serum lipids after acute stroke. *Stroke* 21: 1407-1411;1990.
46. Pedro-Botet J, Send M, et al. Lipoprotein and apolipoprotein profile in men with ischemic stroke: role of lipoprotein(a), triglyceride-rich lipoproteins, and apolipoprotein E polymorphism. *Stroke* 23: 1556-1562; 1992.
47. Shintani S, Kikuchi S, et al. High serum lipoprotein(a) concentrations are an independent risk factor for cerebral infarction. *Stroke* 24: 965-969; 1993.
48. Jovicic A, Ivanisevic V, et al. Lipoprotein(a) in patients with carotid atherosclerosis and ischemic cerebrovascular disorders. *Atherosclerosis* 98: 59-65; 1993.
49. Nagayama M, Shinohara Y, et al. Lipoprotein(a) and ischemic cerebrovascular disease in young adults. *Stroke* 25: 74-78; 1994.
50. Taddei-Peters W, Jurgens G, et al. ELISA quantitation of Lp(a) concentration demonstrates that Lp(a) is an important risk factor in cerebro- and cardiovascular disease. *Abstract Clin Chem* 40/6: 1098-1099; 1994.
51. Seed M, Hopplicher F, et al. Relation of serum lipoprotein(a) concentration and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia. N *Engl J Med* 322: 1494-1499; 1990.
52. Hoff HF, Beck GJ, et al. Serum Lp(a) level as a predictor of vein graft stenosis after coronary artery bypass surgery in patients. *Circulation* 77: 1238-1244; 1988.
53. Solymoss BC, Marcil M, et al. Risk factors of venous aortocoronary bypass graft disease noted at late symptom-directed angiographic study. *Can J Cardiol* 9: 80-84; 1993.
54. Hearn JA, Donohue BC, et al. Usefulness of serum Lp(a) as a predictor of restenosis after percutaneous transluminal coronary angioplasty. *Am J Cardiol* 69: 736-739; 1992.
55. Tenda K~ Saikawa T, et al. The relationship between serum lipoprotein(a) and restenosis after initial elective percutanceus transluminal coronary angioplasty. *Jpn Circ J* 57: 789-795; 1993.
56. Morrisett, JD. Lipoprotein(a) : Structure, metabolism and epidemiology. *Plasma Lipoproteins:* 129; 1987.
57. Dahlen GH, Guyton M et al. Association of levels of lipoprotein(a), plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. *Circulation* 74: 758-765; 1986.
58. Armstrong VW, Cremer P, et al. The association between Lp(a) concentrations and angiographically assessed coronary atherosclerosis. *Atherosclerosis* 62: 249-257; 1986.
59. Schwartzkopff W, Schleicher J, et al. Lipids, lipoproteins, apolipoproteins and other risk factors in Chinese men and women with and without myocardial infarction. *Atherosclerosis* 82: 253-259; 1990.
60. Sandholzer C, Boerwinkle E, et al. Apolipoprotein(a) phenotypes, Lp(a) concentration and plasma lipid levels in relation to coronary heart disease in a Chinese population: evidence for the role of the apo(a) gene in coronary heart disease. *J Clin Invest* 89: 1040-1046; 1992.
61. Farrer M, Game FL, et al. Coronary artery disease is associated with increased lipoprotein(a) concentrations independent of the size of circulating apolipoprotein(a) isoforms. *Arterioscler Thromb* 14: 1272-1283; 1994.
62. Sandkamp M, Funke H, et al. Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. *Clin Chem* 36: 20-23; 1990.

63. Schaefer EJ, Lamon-Fava S, et al. Lipoprotein(a) levels and risk of coronary heart disease in men: the Lipid Research Clinics Coronary Primary Prevention Trial. *JAMA* 271: 999-1003; 1994.
64. Bostom AG, Gagnon DR, et at. A prospective investigation of elevated lipoprotein(a) detected by electrophoresis and cardiovascular disease in women: The Framingham Heart Study. *Circulation* 90: 1688-1695; 1994.
65. Cremer P, Nagel D, et al. Lipoprotein Lp(a) as predictor of myocardial infarction in comparison to fibrinogen, LDL cholesterol and other risk factors: results from the prospective Gottingen Risk Incidence and Prevalence Study (GRIPS). *Europ J Clin -Invest* 24: 444-453; Coronary Heart Disease in Men 1994.
66. Bostom AG, Cupples AL, et al. Elevated Plasma Lipoprotein (a) and Aged 55 Years and Younger. JAMA 276/7: 544-548; 1996.
67. Sigurdsson G, Baldursdottir A, et al. Predictive value of apolipoproteins; in a prospective survey of coronary artery disease in men. *Am J Cardiol* 69: 1251-1254; 1992.
68. Rosengren A, Wilhelmsen L, et al. Lipoprotein(a) and coronary heart disease: a prospective case-control in a general population sample of middle aged men. *Br Med J* 301: 1248-1250; 1990.
69. Wald NJ, Law M, et al. Apolipoproteins and ischemic heart disease: implications for screening. *Lancet* 343: 75-79; 1994.
70. Cantin B, Moorjani S, et al. Lp(a) in ischemic heart disease: the Quebec Cardiovascular Study. J *Am Coll Cardiol* 23: 482A Abstract 837-843; 1994.
71. National Cholesterol Education Program. Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 1993 Bethesda MD, NTH National Heart, Lung and Blood Institute NTH Publication No. 93-3095.
72. Rhoads GG, Dahlen G, Berg K, et al. Lp(a) Lipoprotein as a Risk Factor for Myocardial Infarction, JAMA 256: 2540-2544; 1986.
73. Min WK, Lee TO, Hub JW. Relation Between Lipoprotein(a) Concentrations in Patients with Acute Phase Response and Risk Analysis for Coronary Heart Disease. *Clinical Chemistry* 43: 1891-1895; 1997.
74. Rodriguez CR, Seman LJ, Ordovas JM, et al. Lipoprotein (a) and Coronary Heart Disease. *Chemistry and Physics of Lipids* 67/68: 389-398; 1994.
75. Marcovina SM, Alber JJ, et al. Effect of the Number of Apolipoprotein(a) Kringle 4 Domains on Immunochemical Measurements of Lipoprotein(a). *Clin Chem* 41/2: 246-255; 1995.

The safety data sheet is available upon request.

WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + **P352**: IF ON SKIN: Wash with plenty of soap and water.

P333 + **P313**: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed

P264: Wash thoroughly with plenty of soap and water after handling

P270: Do not eat, drink or smoke when using this product

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P330: If swallowed, rinse mouth

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

ORDERING INFORMATION

KIT		Macra® Lp(a) Test Kit
Catalog No.	Item	Quantity
2339100	Macra® Lp(a) Test Kit	96 Tests

Manufactured

CONTROL + + High Pos or Positive Control

EC REP Authorized Representative

CONTROL + Low Pos or Cut-Off Control

Consult accompanying documents

CONTROL - Negative Control

REF Product Number

CAL Calibrator

LOT Lot

C.F. Coefficient Factor

Use by

RNG Range

Caution, consult accompanying documents

STD Standard

2°C 8°C Store at 2-8ºC

IVD For *In Vitro* Diagnostic use

2°C 30°C Store at 2-30ºC

or Warning

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