DRG® Total human Lipoprotein A (Lp(a)) (EIA-4406)

CE

Revised 13 Jan. 2011 rm (Vers. 2.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE
To quantify total human Lipoprotein A (Lp(a)).

PRINCIPLE OF PROCEDURE
Solid phase capture sandwich ELISA assay using a microwell format.

SHELF LIFE
The expiration date for the package and each component is stated on the label(s). Store all components at 2°-8° degrees C with the exception of the standard, which should be stored frozen.

SAMPLE AND STANDARD DILUTIONS
Dilute each serum or plasma specimen to be tested 1:400 with the Lp(a) specimen diluent provided. (Serum specimens with high Lp(a) levels should be diluted more than 1:400 for accurate Lp(a) determination.).

***NOTE: A pre-dilution using PBS (phosphate buffer) may be done followed by a final dilution in specimen diluents to bring the serum or plasma final dilution to 1:400.**

Construct a standard curve as follows:
a) Perform a series of at least four, twofold dilutions of the 1:400 standard. Use the specimen diluent alone as a blank or zero control.
b) Use the declared value on the vial of Lp(a) standard to calculate the values on the standard curve.

MATERIALS SUPPLIED
1. Goat Anti-Human Lp(a) coated microwell strips 12x8 with plastic frame
2. Lp(a)N Conjugate – 12mL
3. Lp(a) standard (diluted 1:400) – 1 mL
4. TMB/peroxide substrate color developer – 12mL
5. Lp(a) specimen diluent - 60mL
6. Sulfuric acid termination reagent (0.5N) –12mL
7. 15 X Wash buffer concentrate – 60mL

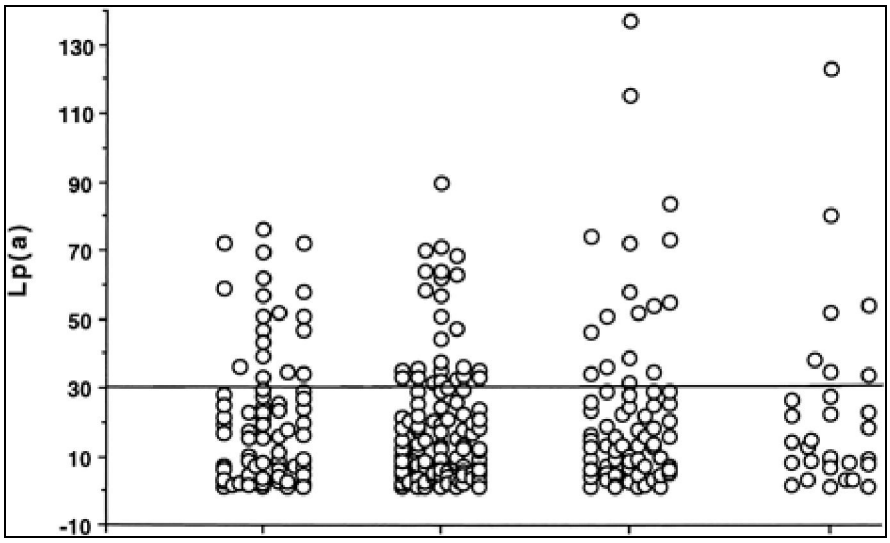
LIMITATIONS OF THE PROCEDURE
FOR RESEARCH USE ONLY.

ASSAY PROCEDURE

**Allow each reagent to reach room temperature before use.*

1. Add 100 µL of *diluted* specimen or standard to each microwell
2. Incubate at room temperature for 60 minutes
3. Decant and wash each microwell four times with wash buffer (dilute buffer 1:15 with reagent grade water)
4. Add 100 µL of anti-human Apo B-100 conjugate to each well
5. Incubate at room temperature for 60 minutes
6. Decant and wash as in step 3
7. Add 100 µL of TMB/peroxide substrate II and incubate at room temperature for 30 minutes
8. Terminate the reaction with 100uL of 0.5N sulfuric acid
9. Zero the microwell reader at 450nm using the specimen diluent zero control well
10. Determine the optical density (O.D.) of the remaining wells
11. Construct a standard curve using the O.D. values obtained for each of the standards
12. Interpolate the unknowns from the standard curve

Table 1. Lp(a) levels (mg/dl) in centenarians and controls :



Centenarians (*n*=75)		<65 years, randomly selected (*n*=114)	>65 years, randomly selected (*n*=73)	>60 years, healthy selected (*n*=30)
Age range (in years)	100–106	8–64	65–98	61–80
Age mean	100.9 ± 1.4	35.8 ± 11.8	83.5 ± 7.6	71.4 ± 5.5
Lp(a) average	22.4	19.3	23.8	23.0
Lp(a) median	17.2	12.5	15.2	14.2
Lp(a) range	1–76	1–90	1–137	1–123
Log Lp(a)(±SD)	1.11 ± 0.52	1.06 ± 0.48	1.13 ± 0.51	1.12 ± 0.51
% subjects with				
Lp(a) >30 mg/dl	25.3	22.8	23.3	23.3
% subjects with				
Lp(a) <30 mg/dl	74.7	77.2	76.7	76.7

Table is from “Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors” in the Faseb Journal 1998; 12:433-437

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