

Total Human Lipoprotein a (Lp(a)) ELISA Assay

Intended Use:

To quantitate total human Lipoprotein A (Lpa)

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°C with the exception of the standard, which should be stored frozen.

Patient 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 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:

Goat Anti-Human Lp(a) coated microwell strips 12x8 with plastic frame

Lp(a)N Conjugate -12mL

Lp(a) standard (diluted 1:400) – 1 mL

TMB/peroxide substrate color developer–12mL

Lp(a) specimen diluent - 60mL

Sulfuric acid termination reagent (0.5N) –12mL

15 X Wash buffer concentrate – 60mL

Limitations of the Procedure:

No single assay should be used as the only basis for arriving at a diagnostic conclusion. For research use only.

Dynamic Range:

3 µg/dL-405µg/dL

Reproducibility:

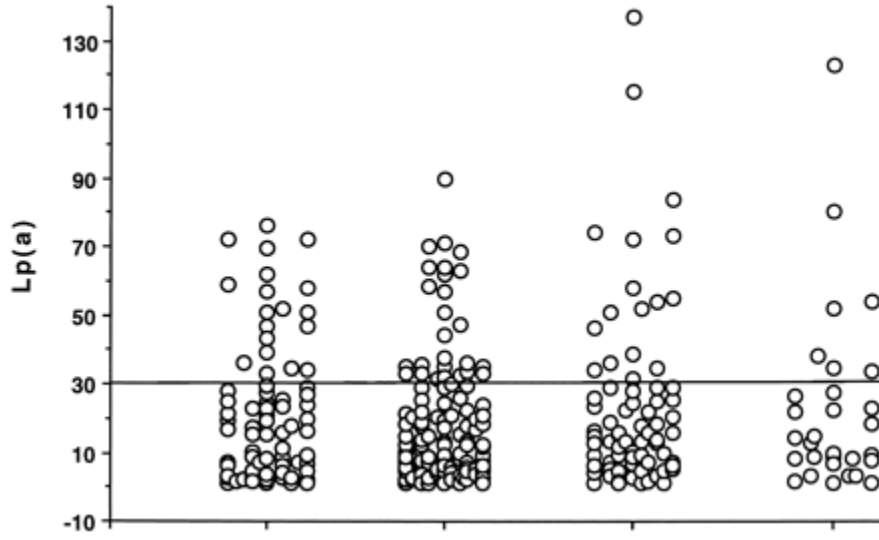
C.V. 4%-8% depending upon the region of the standard curve.

Assay Procedure:

*Allow each reagent to reach room temperature before use

1. Add 100uL of *diluted* specimen or standard to each microwell
2. Incubate at room temperature for 60 minutes
3. Decant and wash each microwell five times with wash buffer (dilute buffer 1:15 with reagent grade water)
4. Add 100uL 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 100uL of TMB/peroxide substrate 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)(\bar{x}
 \pm SD)

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