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# Total Human Lipoprotein a



Test	Lp(a) ELISA	
Method	ELISA: Enzyme Linked Immunosorbent Assay	
Principle	ELISA – Solid Phase, Sandwich ELISA	
<b>Detection Range</b>	$3 \mu g/dL - 405 \mu g/dL$	
Sample	10ul Serum	
Specificity	90%	
Sensitivity	3 μg /dL	
<b>Total Time</b>	~150 min	
Shelf Life	12 -18 Months	

DAI Code # 9

#### **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°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 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 **Store at -20 °C**
- 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:**

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

## **Dynamic Range:**

 $3 \mu g/dL - 405 \mu g/dL$ 

## Reproducibility:

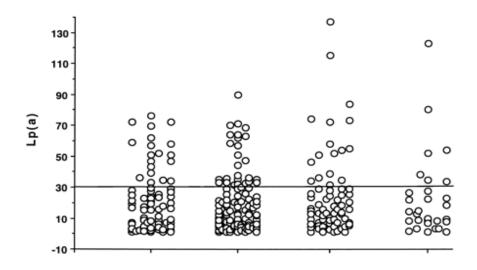
C.V. 4%-8% depending upon the region of the standard curve.

DAI Code # 9 2

### **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



DAI Code # 9

	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	$100.9 \pm 1.4$	$35.8 \pm 11.8$	$83.5 \pm 7.6$	$71.4 \pm 5.5$
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
$Log Lp(a)(\overline{\times}\pm SD)$	$1.11 \pm 0.52$	$1.06 \pm 0.48$	$1.13 \pm 0.51$	$1.12 \pm 0.51$
% 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

Date Adopted	Reference No.
2008-04-28	DA-Lp (a)-2009

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DAI Code # 9