Lipoprotein(a) (Lp(a)) Total ELISA

Catalog Number: LPA31-K01
1 x 96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
Intended Use:

The Eagle Biosciences Lipoprotein(a) (Lp(a)) ELISA assay kit is intended for the quantitative determination of Lp(a) in plasma by enzyme linked immunoassay (ELISA). The Lipoprotein(a) (Lp(a)) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

Assay Background:

Lp(a), unlike Apo AI or Apo B, whose levels vary as a result of diet, exercise, etc. is predominantly a genetic trait whose level remains more or less constant after puberty. More than 13 phenotypes of Lp(a) have been identified having molecular weight of 300-800 Kd. It is bound to both HDL and LDL. Lp(a) interferes with plasminogen, the clot dissolving enzyme, which binds to the arterial endothelial lining. This in turn contributes to blood clot formation, and over a prolonged period of time would lead to significant damage to the coronary arteries. Levels of greater than 30mg/dl have been demonstrated to independently increase the risk of CHD by six fold.

Principle of Procedure:

The Lipoprotein(a) (Lp(a)) ELISA assay kit determines total human Lp(a) according to the “sandwich” principle. Lp(a) in samples and standards binds to antibodies which are coated to the microtiter plate. After a washing step a peroxidase labeled detection antibody is added. A second washing step is followed by the addition of the substrate which is converted to a colored product by the peroxidase. The reaction is terminated by the addition of an acidic stop solution. The optical densities are read at 450 nm in a microtiter plate reader. The Lp(a) concentration can be calculated from the standard curve.

Materials Provided:

The expiration date for the Lipoprotein(a) (Lp(a)) ELISA assay kit and each component is stated on the label(s). Store all components at 2-8°C with the exception of the Lp(a) standard, which should be stored at -20°C.

- 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

**Reagent and Sample Preparation:**

- Dilute the 15X wash buffer provided 1:15 using one part wash buffer concentrate and 14 parts reagent grade water.

- 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.

- Perform a series of at least four, twofold dilutions of the 1:400 standard to construct the standard curve. Use specimen diluent alone as a blank or zero control.

**Assay Procedure:**

Allow each reagent of the Lipoprotein(a) (Lp(a)) ELISA assay kit 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

Calculations:

For calculating the results we recommend using the 4-parameter algorithm. First, zero the microwell reader at 450 nm using the blank control well. If this algorithm is not available a “point to point” or a “spline” function can be used.

Manual processing of results: Correct each absorbance value by subtracting the background absorbance (blank). Estimate the mean value for each duplicate.

Construct a standard curve by plotting the mean absorbance value for each standard (y-axis) against the corresponding concentration (x-axis) on semi-log graph paper and read the concentration of unknowns off the curve.

The curve given above is only for demonstration. It must not be used for calculation of your samples.
**Dynamic Range:**

The dynamic range of the Lipoprotein(a) (Lp(a)) ELISA assay kit is 3 µg/dL-405µg/dL.

**Reproducibility:**

The Lipoprotein(a) (Lp(a)) ELISA assay kit was found to have a reproducibility with a C.V. 4% - 8% depending on region of the standard curve.
### Table 1. Lp(a) levels (mg/dl) in centenarians and controls

<table>
<thead>
<tr>
<th></th>
<th>Centenarians (n=75)</th>
<th>&lt;65 years, randomly selected (n=114)</th>
<th>&gt;65 years, randomly selected (n=73)</th>
<th>&gt;60 years, healthy selected (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range (in years)</td>
<td>100–106</td>
<td>8–64</td>
<td>65–98</td>
<td>61–80</td>
</tr>
<tr>
<td>Age mean</td>
<td>100.9 ± 1.4</td>
<td>35.8 ± 11.8</td>
<td>83.5 ± 7.6</td>
<td>71.4 ± 5.5</td>
</tr>
<tr>
<td>Lp(a) average</td>
<td>22.4</td>
<td>19.3</td>
<td>23.8</td>
<td>23</td>
</tr>
<tr>
<td>Lp(a) median</td>
<td>17.2</td>
<td>12.5</td>
<td>15.2</td>
<td>14.2</td>
</tr>
<tr>
<td>Lp(a) range</td>
<td>1–76</td>
<td>1–90</td>
<td>1–137</td>
<td>1–123</td>
</tr>
<tr>
<td>Log Lp(a)(x±SD)</td>
<td>1.11 ± 0.52</td>
<td>1.06 ± 0.48</td>
<td>1.13 ± 0.51</td>
<td>1.12 ± 0.51</td>
</tr>
<tr>
<td>% subjects with Lp(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30 mg/dl</td>
<td>25.3</td>
<td>22.8</td>
<td>23.3</td>
<td>23.3</td>
</tr>
<tr>
<td>&lt;30 mg/dl</td>
<td>74.7</td>
<td>77.2</td>
<td>76.7</td>
<td>76.7</td>
</tr>
</tbody>
</table>

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
**Warranty Information**

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

*For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.*