Lipoprotein (a) Assay Kit

Configuration
The Diazyme Lipoprotein (a) reagent is provided in bulk and the following kit configurations:

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ131A-K</td>
<td>R1: 1 x 60 mL</td>
</tr>
<tr>
<td></td>
<td>R2: 1 x 20 mL</td>
</tr>
</tbody>
</table>

Intended Use
The Diazyme Lp(a) Assay is intended as a latex particle enhanced immunoturbidimetric assay for the in vitro quantitative determination of lipoprotein(a) [Lp(a)] concentration in human serum or plasma (EDTA) on Clinical Chemistry Systems. The measurement of Lp(a) is useful in evaluating lipid metabolism disorders and assessing atherosclerotic cardiovascular diseases in specific populations, when used in conjunction with clinical evaluation. For in vitro diagnostic use only.

Diazyme Lp(a) Control is intended for use in monitoring the quality control of results obtained with the Diazyme Lp(a) reagents by turbidimetry.

Diazyme Lp(a) standard is intended for use in establishing the calibration curve for the Diazyme Lp(a) reagents by turbidimetry.

Clinical Significance
Lipoprotein (a) is a cholesterol-rich lipoprotein particle found in human serum. There is substantial evidence linking lipoprotein (a) excess to a high risk for premature coronary heart disease (CHD), increased risk of myocardial infarction (MI) and stroke, and restenosis after angioplasty (PTCA) and coronary bypass procedures.\(^1\,^8\) Assessment should be based on Patient history, Clinical findings and other laboratory tests.

Assay Principle
The Diazyme Lipoprotein (a) Assay is based on a latex enhanced immunoturbidimetric assay. Lp(a) in the sample binds to specific anti-Lp(a) antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of Lp(a) in the sample.

Reagent – “Working Solutions”
- **REAGENT 1**: Glycine Buffer Solution
- **REAGENT 2**: Latex particles coated with anti-Lp(a) antibodies

Precautions
**DO NOT INGEST.** Avoid contact with skin and eyes. Contains sodium azide, which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of this reagent. Calibrators and controls are human serum based. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

Reagent Handling
1. The Diazyme Lp(a) Assay **REAGENT** provided is ready to use.
2. Physiological saline is needed to dilute high Lp(a) samples.

Reagent Stability and Storage
The Diazyme Lp(a) Assay **REAGENTS, CALIBRATORS, and CONTROLS** should be stored at 2-8°C. **DO NOT FREEZE.** The **REAGENTS, CALIBRATORS, and CONTROLS** are stable when stored as instructed until the expiration date on the label. Do not mix **REAGENTS** from different lots.

Specimen Collection and Handling
Serum or EDTA plasma samples can be used for the Lp(a) assay. Analyze fresh specimens if possible. Repeated freeze/thaw cycles should be avoided to minimize potential protein degradation.

Materials Provided
Please see the “Reagents – “Working Solutions”” section.

Materials Required but not Provided
An analyzer capable of dispensing two reagents and of measuring absorbance at 700 nm with temperature control (37°C). **CONTROLS** for validating the performance of the Diazyme Lp(a) Assay **REAGENTS** are provided separately (**REF** DZ131A-CON and DZ131B-CON) and saline for diluting serum samples is not provided.

Assay Procedures
Lp(a) should be measured according to the specific application parameters for the specific chemistry analyzer. Below is a general example of the assay procedure as run on the Hitachi 717 analyzer.
1. Incubation of 6 μL sample with 225 μL **R1** at 37°C for 5 minutes.
2. Addition of 75 μL **R2**.
3. Reading of an absorbance change at 700nm for 4.5 minutes, 20 seconds after the addition of **R2**.
4. Calculation of Lp(a) value with the read absorbance change by interpolation from a calibration curve prepared with calibrators of known concentrations.
Application sheets for use of Diazyme Lp(a) Assay on other automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

**Calibration**

Five levels of lyophilized calibrators ([REF] DZ131A-CAL) are available and must be reconstituted with water prior to use. Use saline and the provided calibrator levels 1-5 for calibration. The reconstituted calibrators are stable for at least 2 weeks when stored at 2-8°C.

Five levels of liquid stable calibrators are also available ([REF] DZ131B-CAL).

**Quality Control**

We recommend that each laboratory use Lp(a) controls to validate the performance of Lp(a) [REAGENTS]. A set of normal and abnormal ranges of Lp(a) [CONTROLS] is available from Diazyme Laboratories ([REF] DZ131A-CON and DZ131B-CON). The range of acceptable control limits should be established by individual laboratories.

**Results**

Results are printed out in mg/dL. Note: Samples with values greater than 100 mg/dL should be diluted 1:2 with saline and re-assayed. Multiply results by 2.

**Reference Range**

Studies have shown the expected range for Lp(a) has been reported to be between 10 and 30 mg/dL.9,10 Some studies have indicated that Lp(a) concentrations in African Americans may differ from Caucasians with African American ranges higher.12,13

Each laboratory, however, is recommended to establish a range of normal values for the population in their region. Lp(a) values should be interpreted in conjunction with clinical evaluation and other lipoprotein tests when assessing atherosclerotic cardiovascular disease in specific populations.

**Limitations**

1. Harmonization efforts for Lp(a) assay methods have suggested an impact of Apo A size heterogeneity on Lp(a) measurement methods.11,12 The effects of the impact of Apo A size have not been assessed for this assay.
2. A sample with a Lp(a) level exceeding the linearity limit of 100 mg/dL should be diluted with saline and re-assayed incorporating the dilution factor in the calculation of the value.
3. Store the reagents at 2-8°C. Do not freeze the reagents.
4. Assessment should be based on Patient history, Clinical findings and other laboratory tests.

**Performance Characteristics**

The assay performance was established on Hitachi 717 with a 6-point calibration using saline and separately provided calibrator levels 1-5. Results obtained from individual laboratories may vary.

**Accuracy**

Correlation studies were performed by testing 76 serum ranging from 5.21 to 94.83 mg/dL in comparison with an existing commercial Lp(a) assay method.

The correlation coefficient between the two methods was 0.9983, slope was 0.9895, and y intercept was 0.0279.

**Precision**

The precision of the Diazyme Lp(a) Enzymatic Assay was evaluated according to Clinical and Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study, three levels of serum specimens containing about 17.2, 43.2, and 70.0 mg/dL. Lp(a) respectively are tested with 2 runs per day with duplicates over 20 working days on Hitachi 717.

**Within Run Precision (S_d)**

<table>
<thead>
<tr>
<th>Level 1: mg/dL Lp(a)</th>
<th>Level 2: mg/dL Lp(a)</th>
<th>Level 3: mg/dL Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Data Points</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>18.3</td>
<td>42.2</td>
</tr>
<tr>
<td>SD (mg/dL)</td>
<td>0.47</td>
<td>0.59</td>
</tr>
<tr>
<td>CV%</td>
<td>2.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**Within-Laboratory Precision (S_Y)**

<table>
<thead>
<tr>
<th>Level 1: mg/dL Lp(a)</th>
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<tr>
<td>SD (mg/dL)</td>
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<td>1.38</td>
</tr>
<tr>
<td>CV%</td>
<td>3.6</td>
<td>3.3</td>
</tr>
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</table>

**Linearity**

The assay has an analytical measuring assay range up to 100 mg/dL.

**Detection Limits**

The limit of detection (LOD) for Lp(a) assay is determined to be 1.14 mg/dL and the limit of Quantitation (LOQ) for Lp(a) assay is 5.44 mg/dL. The assay has measuring range of 5.44 to 100 mg/dL Lp(a).

**Interference**

To determine the level of interference from the substances normally present in the serum, Diazyme Lp(a) Assay is tested with approximately 16 mg/dL Lp(a) (low) and 43 mg/dL Lp(a) (high) serum samples spiked with various concentrations of substances following Clinical and Laboratory Standards Institute (formerly NCCLS) EP7-A “Interference Testing in Clinical Chemistry”: dose-response guidelines.

The following substances normally present in the serum produced less than 10% deviation when tested at levels equal to the concentrations listed below.

<table>
<thead>
<tr>
<th>Interference</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>1000 mg/dL</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>10 mM</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Bilirubin Conjugated</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1000 mg/dL</td>
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</tbody>
</table>
References


