Amplite™ Colorimetric L- Lactic acid (L-Lactate) Assay Kit

**Introduction**

Lactic acid is chiral and has two optical isomers: L-lactic acid and D-lactic acid. Lactate is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH) in the process of metabolism and exercise. Monitoring lactate levels is a good way to evaluate the balance between tissue oxygen demand and utilization and is useful when studying cellular and animal physiology. D-lactate is not metabolized by mammals and its elimination from the body depends mainly on renal excretion. D- and L-lactic acid are found in many fermented milk products such as yoghurt and cheese, and also in pickled vegetables, and cured meats and fish. The D- and L-lactic acid content is a quality indicator of foods, such as egg, milk, fruit juice and wine. Abnormal high concentration of D-lactate in the blood is usually a reflection of bacterial overgrowth in the gastrointestinal tract.

AAT Bioquest’s Amplite™ Lactate Assay Kits (Cat# 13814 and 13815 for L-lactate assay, and Cat # 13810 and 13811 for D-lactate assay) provide both fluorescence and absorbance-based method for detecting either L-lactate or D-lactate in biological samples such as serum, plasma, urine, as well as in cell culture samples. In the enzyme coupled assay, lactate is proportionally related to NADH, which is specifically monitored by a chromogenic NADH sensor. The signal can be easily read by an absorbance microplate reader at the absorbance ratio of \( \frac{A_{575nm}}{A_{605nm}} \). With this Colorimetric Amplite™ L-Lactate Assay Kit, we were able to detect as little as 4 \( \mu \)M L-lactate in a 100 \( \mu \)L reaction volume. It is robust, and can be readily adapted for a wide variety of applications that require the measurement of L-lactate.

**Kit Components**

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A: Enzyme Probe</td>
<td>2 bottles (lyophilized powder)</td>
</tr>
<tr>
<td>Component B: Assay Buffer</td>
<td>1 bottle (10 mL)</td>
</tr>
<tr>
<td>Component C: NAD</td>
<td>1 vial</td>
</tr>
<tr>
<td>Component D: L-Lactate Standard</td>
<td>2.25 mg/vial</td>
</tr>
</tbody>
</table>

**Assay Protocol for One 96-Well Plate**

**Brief Summary**

Prepare L-lactate assay mixture (50 \( \mu \)L) → Add L-lactate standards or test samples (50 \( \mu \)L) → Incubate at room temperature for 30 min ~ 2 hours → Monitor absorbance ratio increase at \( \frac{A_{575nm}}{A_{605nm}} \)

*Note: Thaw one of each kit component at room temperature before starting the experiment.*

1. **Prepare NAD stock solution (100X):**
   
   Add 100 \( \mu \)L of \( \text{H}_2\text{O} \) into the vial of NAD (Component C) to make 100 X NAD stock solutions.

2. **Prepare L-Lactate stock solution:**
   
   Add 200 \( \mu \)L of \( \text{H}_2\text{O} \) or 1xPBS buffer into the vial of L-Lactate Standard (Component D) to make 100 mM D-lactate standard solution.
   
   *Note: The unused L-lactate standard stock solution should be divided into single use aliquots and stored at -20°C.*

3. **Prepare serial dilutions of L-Lactate standard (0 to 1 mM):**
   
   3.1 Add 10 \( \mu \)L of L-Lactate stock solution (from Step 2) into 990 \( \mu \)L PBS buffer to generate 1 mM L-Lactate standard solution.
   
   *Note: Diluted L-Lactate standard solution is unstable, and should be used within 4 hours.*
   
   3.2 Take 200 \( \mu \)L of 1 mM L-Lactate standard solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 \( \mu \)M serial dilutions of L-Lactate standard.
   
   3.3 Add serial dilutions of L-Lactate standard and L-Lactate containing test samples into a white clear bottom 96-well microplate as described in Tables 1 and 2.

**Table 1** Layout of L-Lactate standards and test samples in a white clear bottom 96-well microplate

<table>
<thead>
<tr>
<th>BL</th>
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<th>TS</th>
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<tbody>
<tr>
<td>Lac</td>
<td>Lac</td>
<td>1</td>
<td>1</td>
<td>…</td>
<td>…</td>
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</tbody>
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**References**