**Preparation of Assay Reagents:** (per ~ 95 assays)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled water</td>
<td>12.9 mL</td>
</tr>
<tr>
<td>bottle 1 (buffer)</td>
<td>5 mL</td>
</tr>
<tr>
<td>*bottle 2 (NAD)</td>
<td>1 mL</td>
</tr>
<tr>
<td>bottle 3 (D-GPT)</td>
<td>0.2 mL</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>19.1 mL</strong></td>
</tr>
</tbody>
</table>

*after adding 5.5 mL of distilled water

**Reagent 1 stability:** > 2 days at 4°C

**Reagent 2:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled water</td>
<td>2.2 mL</td>
</tr>
<tr>
<td>bottle 4 (L-LDH)</td>
<td>0.2 mL</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>2.4 mL</strong></td>
</tr>
</tbody>
</table>

**Reagent 2 stability:** > 2 days at 4°C

**Calibrators:**
- K-LATE 1: 0 g/L (use distilled water)
- K-LATE 2: 0.25 g/L L-lactic acid
- K-LATE 3: 0.5 g/L L-lactic acid
- K-LATE 4: 1.0 g/L L-lactic acid

**Assay Parameters:**
- **Assay volumes:** Reagent 1: 0.200 mL
- **Sample:** 0.010 mL
- **Reagent 2:** 0.025 mL
- **Calibrators:** 0, 0.25, 0.5, 1.0 g/L L-lactic acid
- **Reaction time:** 10 min at 37°C
- **Wavelength:** 340 nm
- **Assay type:** endpoint
- **Reaction direction:** increase
- **Linearity:** up to 1 g/L of L-lactic acid

**Use:**
- For the specific measurement of L-lactic acid especially in wines, fruit juices, beverages and food products.
- For specific sample preparation methods refer to the L-Lactic Acid (L-Lactate) Assay Kit (K-LATE) data booklet.

**Assay Principle:**
Conversion of L-lactic acid via the following reactions is directly proportional to the coupled formation of NADH:

\[
\begin{align*}
(1) \quad & \text{L-Lactate} + \text{NAD}^+ & \rightarrow & \text{pyruvate} + \text{NADH} + \text{H}^+ \\
(2) & \text{Pyruvate} + \text{D-glutamate} & \rightarrow & \text{D-alanine} + 2\text{-oxoglutarate}
\end{align*}
\]

**Procedure:**
Prepare the assay reagents and calibrators and use with the K-LATE ChemWell® 2910 assay file.

**L-Lactic Acid (L-Lactate) Assay Kit Components:**

**Bottle 1:** Buffer (25 mL, pH 10.0) plus D-glutamate and sodium azide (0.02% w/v) as a preservative. Stable for > 2 years at 4°C.

**Bottle 2:** NAD+. Stable for > 5 years at -20°C.

**Bottle 3:** D-Glutamate-pyruvate transaminase suspension (1.1 mL). Stable for > 2 years at 4°C.

**Bottle 4:** L-Lactate dehydrogenase suspension (1.1 mL). Stable for > 2 years at 4°C.

**Preparation of Kit Components:**
1. Use the contents of bottle 1 as supplied. Stable for > 2 years at 4°C.
2. Dissolve the contents of bottle 2 in 5.5 mL of distilled water. **Stable for > 1 year at 4°C or > 2 years at -20°C** (to avoid repetitive freeze / thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).
3 & 4. Use the contents of bottles 3 and 4 as supplied. Swirl the bottle to mix contents before use. Stable for > 2 years at 4°C.