Preparation of Assay Reagents: (per ~ 95 assays)

Reagent 1:

<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>Total volume</td>
<td>19.1 mL</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>Total volume</td>
<td>2.4 mL</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

**Requirements:**

- L-Lactic Acid (L-Lactate) Assay Kit (K-LATE) (provides ~ 475 assays).
- Use in association with the L-Lactic Acid (L-Lactate) Assay Kit (K-LATE) product data booklet.

**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:

1. \[ \text{L-Lactate} + \text{NAD}^+ \rightarrow \text{pyruvate} + \text{NADH} + \text{H}^+ \] (L-LDH)
2. \[ \text{Pyruvate} + \text{D-glutamate} \rightarrow \text{D-alanine} + 2\text{-oxoglutarate} \] (D-GPT)

**Procedure:**

Prepare the assay reagents and calibrators and use with the K-LATE ChemWell®-T 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.

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Version 03.2014