**Preparation of Assay Reagents: (per ~ 93 assays)**

**Reagent 1:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled water</td>
<td>12.4 mL</td>
</tr>
<tr>
<td>bottle 1 (buffer)</td>
<td>4 mL</td>
</tr>
<tr>
<td>*bottle 2 (NAD/PVP)</td>
<td>2 mL</td>
</tr>
<tr>
<td>bottle 3 (GOT)</td>
<td>0.2 mL</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>18.6 mL</strong></td>
</tr>
</tbody>
</table>

*after adding 12 mL of distilled water

Reagent 1 stability: > 7 days at 4°C

**Reagent 2:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled water</td>
<td>2.1 mL</td>
</tr>
<tr>
<td>bottle 4 (L-MDH)</td>
<td>0.2 mL</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>2.3 mL</strong></td>
</tr>
</tbody>
</table>

Reagent 2 stability: > 7 days at 4°C

**Calibrators:**

- K-LMALAF 1: 0 g/L (use distilled water)
- K-LMALAF 2: 1.5 g/L L-malic acid
- K-LMALAF 3: 3.0 g/L L-malic acid
- K-LMALAF 4: 6.0 g/L L-malic acid

**Assay Parameters:**

- **Assay volumes:** Reagent 1: 0.200 mL
- **Sample:** 0.003 mL
- **Reagent 2:** 0.020 mL

- **Calibrators:** 0, 1.5, 3.0, 6.0 g/L L-malic acid
- **Reaction time:** 3 min at 37°C
- **Wavelength:** 340 nm
- **Assay type:** endpoint
- **Reaction direction:** increase
- **Linearity:** up to 6 g/L of L-malic acid

**Assay Principle:**

Conversion of L-malic acid via the following reactions is directly proportional to the coupled formation of NADH:

1. \[(L-MDH)\]
   \[\text{L-Malic acid} + \text{NAD}^+ \rightarrow \text{oxaloacetate} + \text{NADH} + \text{H^+}\]

2. \[(GOT)\]
   \[\text{Oxaloacetate} + \text{L-glutamate} \rightarrow \text{2-oxoglutarate} + \text{L-aspartate}\]

**Procedure:**

Prepare the assay reagents and calibrators and use with the K-LMALAF ChemWell® 2910 assay file.

**L-Malic Acid (Analyser Format) Assay Kit Components:**

**Bottle 1:** Buffer (48 mL, pH 10.0) plus L-glutamate and sodium azide (0.02% w/v) as a preservative. Stable for > 2 years at 4°C.

**Bottle 2:** (x2) NAD⁺ and PVP. Freeze-dried powder. Stable for > 5 years at -20°C.

**Bottle 3:** Glutamate-oxaloacetate transaminase suspension (2.5 mL). Stable for > 2 years at 4°C.

**Bottle 4:** L-Malate dehydrogenase suspension (2.5 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 12 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.