Preparation of Assay Reagents: (per ~ 100 assays)

Reagent 1:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled water</td>
<td>15 mL</td>
</tr>
<tr>
<td>bottle 1 (buffer)</td>
<td>3 mL</td>
</tr>
<tr>
<td>*bottle 2 (NADPH)</td>
<td>2 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>20 mL</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>2.4 mL</td>
</tr>
<tr>
<td>bottle 3 (GlDH)</td>
<td>0.18 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>2.58 mL</td>
</tr>
</tbody>
</table>

Reagent 2 stability: > 7 days at 4°C

Reagent 3:

<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 (urease)</td>
<td>0.45 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>2.55 mL</td>
</tr>
</tbody>
</table>

Reagent 3 stability: > 2 days at 4°C

Calibrators:

- K-LARGE Urea 1: 0 g/L (use distilled water)
- K-LARGE Urea 2: 0.025 g/L urea
- K-LARGE Urea 3: 0.05 g/L urea
- K-LARGE Urea 4: 0.1 g/L urea

Assay Parameters:

- Assay volumes: Reagent 1: 0.200 mL
  Sample: 0.010 mL
  Reagent 2: 0.025 mL
  Reagent 3: 0.025 mL

- Calibrators: 0, 0.025, 0.05, 0.1 g/L urea
- Reaction time: 5 min at 37°C plus 6 min at 37°C
- Wavelength: 340 nm
- Assay type: endpoint
- Reaction direction: decrease
- Linearity: up to 0.1 g/L of urea

Use:

For the specific measurement of urea especially in wines, fruit juices, beverages and food products.

For specific sample preparation methods refer to the L-Arginine/ Urea/Ammonia Assay Kit (K-LARGE) data booklet.

Assay Principle:

Conversion of urea via the following reactions is directly proportional to the coupled consumption of NADPH:

1. Urea + H₂O → 2NH₃ + CO₂

2. 2-Oxoglutarate + NADPH + NH₄⁺ → L-glutamic acid + NADP⁺ + H₂O

Procedure:

Prepare the assay reagents and calibrators and use with the K-LARGE (UREA) ChemWell® 2910 assay file.

L-Arginine/Urea/Ammonia Assay Kit Components:

Bottle 1: Buffer (18 mL, pH 8.0) plus 2-oxoglutarate and sodium azide (0.02% w/v) as a preservative. Stable for > 2 years at 4°C.

Bottle 2: NADPH. Stable for > 5 years at -20°C.

Bottle 3: Glutamate dehydrogenase suspension (1.1 mL). Stable for > 2 years at 4°C.

Bottle 4: Urease solution (2.7 mL). Stable for > 2 years at -20°C.

Preparation of Kit Components:

1 & 3. Use the contents of bottles 1 and 3 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).

4. Use the contents of bottle 4 as supplied. Stable for > 2 years at -20°C.