**TECHNICAL INFORMATION**

Catalog Number: 101202, 101203, 191498

**Uricase**

**CAS #:** 9002-12-4

**Unit Definition:** 1 unit oxidizes 1 umole of uric acid/minute at pH 8.5, 25°C.

**Description:** Uricase, or urate oxidase, is a copper-containing enzyme catalyzing specifically the oxidation of uric acid. It catalyzes the reaction of uric acid in accordance with the following reaction:

\[
\text{Uric Acid} + 2\text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}.
\]

Uricase is found in the kidneys, livers and spleens of almost all mammals, except in human beings and anthropoidea. It is also known that uricase exists in various kinds of bacteria.

**Optimum pH:** Activation maximum at pH 8.5. Activity is scarcely observed at pH below 5.0 or over 11.0. Stable within the pH range of 7.0 to 11.0

**Optimum Temperature:** Optimum temperature for activity is approximately 40°C.

**Inhibition:** Metallic iron carriers as Cu++ potentially reduce or inhibit the activity. The enzyme activity if inhibited by cyanide ion.

**Stabilizing Agents:** Chelate compounds such as EDTA work as a potential stabilizing agent for uricase.

**Assay**

**Method:** The disappearance of uric acid is followed spectrophotometrically at 290 μm.3 Allantoin shows no absorption at this wave length.

**Reagents**

Enzyme: 1 mg/ml in 0.1 M borate buffer, pH 8.5

Substrate: 20 mg of uric acid (MP # 103215) per liter of water. (Warm to dissolve; make up on the day used.) For use, dilute with an equal volume of 0.2 M borate buffer, pH 8.5

**Procedure:**

To control cuvette add the following in order:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>0.1% KCN</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Water</td>
<td>0.3 ml</td>
</tr>
<tr>
<td>Substrate</td>
<td>2.0 ml</td>
</tr>
</tbody>
</table>

With spectrophotometer at 290 μm adjust optical density to 0.500. To test cuvette add:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Water</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Enzyme at zero time</td>
<td>0.5 ml</td>
</tr>
</tbody>
</table>
Absorbancy readings are taken every minute for 6 or 7 minutes and the rate of absorbancy decrease per minute is determined.

*Calculation:* The molar absorbancy index of uric acid is $1.22 \times 10^4$ cm$^{-1}$.

$$\text{units/mg weight} = \frac{\Delta A/\text{min} \times 1000}{1.22 \times 10^4 \times \text{mg/ml reaction mixture}}$$

Assay of purified uricase suspension is the same except that the enzyme, diluted approximately 1 to 200, can be omitted from the blank, hence KCN inactivation is unnecessary.

$$\text{units/ml} = \frac{\Delta A/\text{min} \times 1000 \times 3 \,(\text{ml}) \times \text{dilution factor}}{1.22 \times 10^4 \times 0.5 \,(\text{ml})}$$

**Availability:**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>101202</td>
<td>Uricase, from beef kidney, activity approximately 4.5 units/gm</td>
<td>1 U, 3 U, 5 U, 25 U, 100 U</td>
</tr>
<tr>
<td>101203</td>
<td>Uricase, from <em>Candida utilis</em>, activity not less than 3 units/mg protein</td>
<td>5 U, 10 U, 25 U, 50 U, 100 U</td>
</tr>
<tr>
<td>191498</td>
<td>Uricase, from <em>Bacillus</em> sp., activity approximately 4 units/mg solid</td>
<td>5 U, 10 U, 25 U, 50 U</td>
</tr>
</tbody>
</table>

**References:**

– *Merck Index* 12th Ed No10015