SIGMA QUALITY CONTROL TEST
PROCEDURE

Enzymatic Assay of XANTHINE OXIDASE
(EC 1.1.3.22)

PRINCIPLE:

Xanthine + H₂O + O₂ → Uric Acid + H₂O₂

CONDITIONS:  T = 25°C, pH = 7.5, A₂₉₀nm, Light Path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  50 mM Potassium Phosphate Buffer, pH 7.5 at 25°C
   (Prepare 200 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.  Adjust to pH 7.5 at 25°C with 1 M KOH.)

B.  0.15 mM Xanthine Solution
   (Prepare 100 ml by initially dissolving Xanthine, Sigma Prod. No. X-0626, in a minimal volume of NaOH.  Add approximately 90 ml of deionized water. Adjust to pH 7.5 at 25°C with either 1 M NaOH or 1 M HCl.  Dilute to a final volume of 100 ml.  PREPARE FRESH.)

C.  Xanthine Oxidase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Xanthine Oxidase in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Reagent B (Xanthine)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>-----</td>
<td>0.10</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the $A_{290nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>0.10</td>
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</table>

Immediately mix by inversion and record the increase in $A_{290nm}$ for approximately 5 minutes. Obtain the $\Delta A_{290nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{290nm}/\text{min Test} - \Delta A_{290nm}/\text{min Blank})(3)(df)}{(12.2)(0.1)}$$

3 = Total volume (in milliliters) of assay  
df = Dilution factor  
12.2 = Millimolar extinction coefficient of Uric Acid at 290 nm  
0.1 = Volume (in milliliter) of enzyme used

units/ml enzyme  
mg solid/ml enzyme

Units/mg solid =  

Units/mg protein =  

UNIT DEFINITION:

One unit will convert 1.0 µmole of xanthine to uric acid per minute at pH 7.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 33 mM potassium phosphate, 0.050 mM xanthine and 0.01 - 0.02 unit xanthine oxidase.
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REFERENCE:


NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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