Enzymatic Assay of GUANASE
(EC 3.5.4.3)

PRINCIPLE:

Guanine + H₂O  \text{Guanase} \rightarrow \text{Xanthine} + \text{NH₃}

Xanthine + H₂O + O₂  \text{XOD} \rightarrow \text{Uric Acid} + \text{H₂O₂}

CONDITIONS:  T = 25°C, pH = 8.0, A₂₉₀nm, Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 25°C with 1 M HCl.)

B. 0.001% (w/v) Guanine Solution (Guanine)
(Prepare by dissolving 10 mg of Guanine, Sigma Prod. No. G-0381, in 10 ml of 1 M NaOH. Then add 1 ml of the Guanine Solution to 100 ml of Reagent A. Adjust to pH 8.0 at 25°C with either 1 M HCl or 1 M NaOH, if necessary.)

C. Xanthine Oxidase Enzyme Solution (XOD)
(Prepare a solution containing 0.3 unit/ml of Xanthine Oxidase, Sigma Prod. No. X-1875, in cold deionized water.)

D. Guanase Enzyme Solution (Guanase)
(Immediately before use, prepare a solution containing 0.10 - 0.15 unit/ml of Guanase in cold deionized water.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Guanine)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent C (XOD)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Enzymatic Assay of GUANASE
(EC 3.5.4.3)

PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the A\textsubscript{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 D (Guanase)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A\textsubscript{290nm} for approximately 10 minutes. Obtain the r A\textsubscript{290nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r A\textsubscript{290nm}/min Test - r A\textsubscript{290nm}/min Blank)(3)(df)}{(12.2)(0.1)}
\]

3 = Total volume (in milliliters) of assay
df = Dilution factor
12.2 = Millimolar extinction coefficient\(^1\) of Uric Acid at 290 nm
0.1 = Volume (in milliliter) of enzyme used

\[
\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]

UNIT DEFINITION:

One unit will deaminate 1.0 µmole of guanine to xanthine per minute at pH 8.0 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 93 mM Tris, 0.0009% (w/v) guanine, 62 mM sodium hydroxide, 0.03 unit xanthine oxidase, and 0.01 - 0.015 unit guanase.

REFERENCES:


Enzymatic Assay of GUANASE
(EC 3.5.4.3)

NOTES:

1. The millimolar extinction coefficient was experimentally determined by Sigma.

2. Xanthine Oxidase Unit Definition: One unit will convert 1.0 µmole of xanthine to uric acid per minute at pH 7.5 at 25°C.

3. This assay is based on the cited references.

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

This procedure is for informational purposes. For a current copy of Sigma’s quality control procedure contact our Technical Service Department.