Enzymatic Assay of L-GLUTAMATE OXIDASE
(EC 1.4.3.11)

**PRINCIPLE:**

\[
L-\text{Glutamate} + O_2 + H_2O \xrightarrow{\text{L-GLUTAMATE OXIDASE}} a\text{-Ketoglutaric Acid} + NH_3 + H_2O_2
\]

\[
2H_2O_2 + H_2O \xrightarrow{\text{Catalase}} 2H_2O + O_2
\]

**CONDITIONS:**  \( T = 30^\circ C, \)  \( pH = 7.4, \)  \( A_{316nm}, \)  \( \text{Light path} = 1 \text{ cm} \)

**METHOD:**  Colorimetric

**REAGENTS:**

A. 100 mM Potassium Phosphate Buffer, pH 7.4 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.4 at 30°C with 1 M KOH.)

B. 100 mM L-Glutamate Solution (L-Glu)
(Prepare 2 ml in deionized water using L-Glutamic Acid, Monosodium Salt, Sigma Prod. No. G-1626.)

C. 25\% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 5 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100\% (w/v), Sigma Stock No. 490-10.)

D. 1 M Acetate Buffer, pH 5.0 at 37°C (Acetate)
(Prepare 25 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.0 at 37°C with 1 M HCl.)

E. 0.10\% (w/v) 3-Methyl-2-Benzothiazolinone Hydrazone Solution (MBTH)
(Prepare 10 ml in deionized water using 3-Methyl-2-Benzothiazolinone Hydrazone, Hydrochloride, Hydrate, Sigma Prod. No. M-8006.)

F. 2.6 mM a-Ketoglutaric Acid Solution (KG)
(Prepare 1 ml in Reagent A using a-Ketoglutaric Acid, Disodium Salt, Sigma Prod. No. K-3752.)
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**REAGENTS:**  (continued)

G. Catalase Enzyme Solution (Catalase)  
(Immediately before use, prepare a solution containing 300 units/ml of Catalase, Sigma Prod. No. C-9322, in cold deionized water.)

H. \textit{L}-Glutamate Oxidase Enzyme Solution (L-Glu Ox)  
(Immediately before use, prepare a solution containing 0.05 - 0.10 unit/ml of \textit{L}-Glutamate Oxidase in cold Reagent A.)

**PROCEDURE:**

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.70</td>
<td>0.80</td>
</tr>
<tr>
<td>Reagent G (Catalase)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent B (L-Glu)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 30°C. Then add:

Reagent H (L-Glu Ox)  
0.10  

Immediately mix by swirling and incubate at 30°C for exactly 20 minutes with shaking. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (TCA)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Acetate)</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Reagent E (MBTH)</td>
<td>0.80</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 50°C for 30 minutes. Then allow to stand at room temperature for 20 minutes. Transfer the solutions to suitable cuvettes and record the $A_{316\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

**COLORIMETRIC ASSAY:**

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (KG)</td>
<td>0.02</td>
<td>0.04</td>
<td>0.06</td>
<td>0.08</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.98</td>
<td>0.96</td>
<td>0.94</td>
<td>0.92</td>
<td>0.90</td>
<td>1.00</td>
</tr>
</tbody>
</table>
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COLORIMETRIC ASSAY:  (continued)

Mix by swirling and incubate at 30°C for exactly 20 minutes with shaking. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (TCA)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Acetate)</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Reagent E (MBTH)</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 50°C for 30 minutes. Then allow to stand at room temperature for 20 minutes. Transfer the solutions to suitable cuvettes and record the A<sub>316nm</sub> for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:

\[ r \text{ A}_{316\text{nm}} \text{ Standard} = \text{A}_{316\text{nm}} \text{ Standard} - \text{A}_{316\text{nm}} \text{ Standard Blank} \]

Prepare a standard curve by plotting the \text{A}_{316\text{nm}} for the Standard versus µmoles of α-Ketoglutarate.

Sample Determination:

\[ r \text{ A}_{316\text{nm}} \text{ Sample} = \text{A}_{316\text{nm}} \text{ Test} - \text{A}_{316\text{nm}} \text{ Blank} \]

Determine the µmoles of α-Ketoglutarate produced using the Standard curve.

\[
\text{Units/ml enzyme} = \frac{\text{µmoles of α-Ketoglutarate produced}}{\text{(df)}} \times \frac{\text{(20)(0.1)}}{\text{Units/ml enzyme}}
\]

\[ \text{df} = \text{Dilution factor} \]

\[ 20 = \text{Time (in minutes) of assay as per the Unit Definition} \]

\[ 0.1 = \text{Volume (in milliliter) of enzyme used in assay} \]

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

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

One unit will form 1.0 µmole of α-ketoglutaric acid from L-glutamic acid per minute at pH 7.4 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 80 mM potassium phosphate, 10 mM L-glutamic acid, 30 units catalase, and 0.005 - 0.01 unit L-glutamate oxidase.

REFERENCES:

Soda, K. (1968) Analytical Biochemistry 25, 228-235


NOTES:

1. Catalase Unit Definition: One unit will decompose 1.0 µmole of H₂O₂ per minute at pH 7.0 at 25°C, while the H₂O₂ concentration falls from 10.3 to 9.2 mM.

2. This assay is based on the cited references.

3. 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.