Enzymatic Assay of L-GLUTAMATE OXIDASE (EC 1.4.3.11)

PRINCIPLE:

\[\text{L-Glutamate} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{L-Glutamate Oxidase}} \text{a-Ketoglutaric Acid} + \text{NH}_3 + \text{H}_2\text{O}\]

\[2 \text{H}_2\text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Catalase}} 2 \text{H}_2\text{O} + \text{O}_2\]

CONDITIONS: T = 30°C, pH = 7.4, A$_{316nm}$, Light path = 1 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. 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.)

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).)

D. 1 M Acetate Buffer, pH 5.0 at 37°C (Acetate) (Prepare 25 ml in deionized water using Sodium Acetate, Trihydrate. 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.)

F. 2.6 mM a-Ketoglutaric Acid Solution (KG) (Prepare 1 ml in Reagent A using a-Ketoglutaric Acid, Disodium Salt.)
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**REAGENTS:** (continued)

G. Catalase Enzyme Solution (Catalase)  
(Immediately before use, prepare a solution containing 300 units/ml of Catalase, in cold deionized water.)

H. \( \alpha \)-Glutamate Oxidase Enzyme Solution (L-Glu Ox)  
(Immediately before use, prepare a solution containing 0.05 - 0.10 unit/ml of \( \alpha \)-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:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (L-Glu Ox)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (TCA)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Acetate)</td>
<td>1.90</td>
</tr>
<tr>
<td>Reagent E (MBTH)</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>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>
</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>
</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>
</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 $A_{316}$ for the Standards and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:
Standard Curve:

\[ r \ A_{316} \text{ Standard} = A_{316} \text{ Standard} - A_{316} \text{ Standard Blank} \]

Prepare a standard curve by plotting the $A_{316}$ for the Standard versus µmoles of α-Ketoglutarate.

Sample Determination:

\[ r \ A_{316} \text{ Sample} = A_{316} \text{ Test} - A_{316} \text{ Blank} \]

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

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

\[ \text{df} = \text{Dilution factor} \]
\[ 20 = \text{Time (in minutes) of assay as per the Unit Definition} \]
\[ 0.1 = \text{Volume (in milliliter) of enzyme used in assay} \]

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

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

This procedure is for informational purposes.