Enzymatic Assay of GLUTAMIC-PYRUVIC TRANSAMINASE (EC 2.6.1.2)

**PRINCIPLE:**

L-Alanine + α-Ketoglutaric Acid $\xrightarrow{\text{GPT}}$ Pyruvate + L-Glutamate

Pyruvate + β-NADH $\xrightarrow{\text{Lactic Acid Dehydrogenase}}$ Lactate + β-NAD

Abbreviations used:

GPT = Glutamic-Pyruvic Transaminase

β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

β-NAD = β-Nicotinamide Adenine Mononucleotide

**CONDITIONS:** $T = 37^\circ C$, pH = 7.4, $A_{340\text{nm}}$, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Tris Buffer, pH 7.4 at 37°C.
   (Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.4 at 37°C with 1 M HCl.)

B. 100 mM α-Ketoglutaric Acid Solution (α-KGA)
   (Prepare 10 ml in Reagent A using α-Ketoglutaric Acid, Monosodium Salt, Prod. No. K-1875.)

C. 1 mM L-Alanine Solution
   (Prepare 10 ml in Reagent A using L-Alanine, Prod. No. A-7627.)

D. 6.4 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
   (Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Preweighed Vial, Stock No. 340-110, in the appropriate volume of Reagent A.)
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REAGENTS: (continued)

E. Lactic Dehydrogenase Enzyme Solution (LDH)
(Immediately before use, prepare a solution containing 400 - 600 units/ml in cold deionized water using Lactic Dehydrogenase, Prod. No. L-2500.)

F. Glutamic-Pyruvic Transaminase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 units/ml of Glutamic-Pyruvic Transaminase in cold deionized water.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>18.5</td>
</tr>
<tr>
<td>Reagent B (α-KGA)</td>
<td>3.0</td>
</tr>
<tr>
<td>Reagent C (L-Alanine)</td>
<td>6.0</td>
</tr>
<tr>
<td>Reagent D (β-NADH)</td>
<td>0.5</td>
</tr>
<tr>
<td>Reagent E (LDH)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Mix and adjust to pH 7.4 at 37°C with 1 M NaOH or 1 M HCl, if necessary.

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>Reaction Cocktail</td>
<td>2.90</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Monitor the A$_{340}$nm 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>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A$_{340}$nm for approximately 5 minutes. Obtain the ΔA$_{340}$nm/minute using the maximum linear rate for both the Test and Blank.
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CALCULATIONS:

\[
\text{Units/mg enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(df)}{(6.22)(0.1)} \\
3 = \text{Total volume (in milliliters) of assay} \\
df = \text{Dilution factor} \\
6.22 = \text{Millimolar extinction coefficient of NADH at 340nm}
\]

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

UNIT DEFINITION:

One unit will convert 1.0 \( \mu \)Mole of \( \alpha \)-ketoglutarate to L-glutamate per minute at pH 7.4 at 37\(^\circ\)C, in the presence of L-alanine.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 93 mM Tris, 10 mM \( \alpha \)-ketoglutarate, 200 mM L-alanine, 0.11 mM \( \beta \)-NADH, 13 - 20 units lactic dehydrogenase and 0.03 - 0.06 units glutamic pyruvic transaminase.

NOTES:

1. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 \( \mu \)mole of pyruvate to L-lactate per minute at pH 7.5 at 37\(^\circ\)C.

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

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