Enzymatic Assay of GLUTAMIC-PYRUVIC TRANSAMINASE  
(EC 2.6.1.2)

PRINCIPLE:

\[ \text{L-Alanine} + \text{a-Ketoglutaric Acid} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L-Glutamate} \]

\[ \text{Pyruvate} + \beta-\text{NADH} \xrightarrow{\text{Lactic Acid Dehydrogenase}} \text{Lactate} + \beta-\text{NAD} \]

Abbreviations used:
GPT = Glutamic-Pyruvic Transaminase
\( \beta-\text{NADH} = \beta-\text{Nicotinamide Adenine Dinucleotide, Reduced Form} \)
\( \beta-\text{NAD} = \beta-\text{Nicotinamide Adenine Dinucleotide} \)

CONDITIONS: \( T = 37\degree C, \text{ pH} = 7.4, A_{340} \text{nm}, \text{ Light path} = 1 \text{ cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris Buffer, pH 7.4 at 37\degree C.  
(Prepare 100 ml in deionized water using Trizma Base, Adjust to pH 7.4 at 37\degree C with 1 M HCl.)

B. 100 mM a-Ketoglutaric Acid Solution (a-KGA)  
(Prepare 10 ml in Reagent A using a-Ketoglutaric Acid, Monosodium Salt.)

C. 1 mM L-Alanine Solution  
(Prepare 10 ml in Reagent A using L-Alanine)

D. 6.4 mM \( \beta-\text{Nicotinamide Adenine Dinucleotide, Reduced Form Solution (\( \beta-\text{NADH} \))} \)  
(Dissolve the contents of one 10 mg vial of \( \beta-\text{Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Preweighed Vial, in the appropriate volume of Reagent A.} \)
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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.)

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 (a-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>
<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>

Equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

\[
\text{Units/mg enzyme} = \frac{(r_{A_{340nm}}/\text{min Test} - r_{A_{340nm}}/\text{min Blank})(3)(df)}{(6.22)(0.1)}
\]

3 = Total volume (in milliliters) of assay  
\(df\) = Dilution factor  
6.22 = Millimolar extinction coefficient of NADH at 340nm

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

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

UNIT DEFINITION:

One unit will convert 1.0 µMole of a-ketoglutarate to L-glutamate per minute at pH 7.4 at 37°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 a-ketoglutarate, 200 mM L-alanine, 0.11 mM β-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 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

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

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