Enzymatic Assay of \( \alpha \)-KETOGLUTARATE DEHYDROGENASE

PRINCIPLE:
\[
\alpha \text{-KGA} + \text{Coenzyme A} + \beta \text{-NAD} \xrightarrow{\alpha \text{-KGDH}} \text{Succinyl-CoA} + \beta \text{-NADH} + \text{CO}_2
\]

Abbreviations:
- \( \alpha \text{-KGDH} \) = \( \alpha \)-Ketoglutarate Dehydrogenase
- \( \beta \text{-NAD} \) = \( \beta \)-Nicotinamide Adenine Dinucleotide, Oxidized Form
- \( \beta \text{-NADH} \) = \( \beta \)-Nicotinamide Adenine Dinucleotide, Reduced Form
- \( \alpha \text{-KGA} \) = \( \alpha \)-Ketoglutaric Acid
- CoA = Coenzyme A

CONDITIONS: \( T = 30^\circ \text{C}, \ pH \ 7.4, \ A_{340nm}, \ \text{Light path} = 1 \ \text{cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 150 mM MOPS HCl Buffer, pH 7.4 at 30°C
   (Prepare 100 ml in deionized water using MOPS, Sodium Salt. Adjust to pH 7.4 at 30°C with 1 M HCl.)

B. 12 mM Magnesium Chloride Solution (MgCl\(_2\))
   (Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate.)

C. 0.6 mM Calcium Chloride Solution (CaCl\(_2\))
   (Prepare 100 ml in deionized water using Calcium Chloride, Dihydrate.)

D. 18 mM Cocarboxylase Solution (Cocarboxylase)
   (Prepare 10 ml in deionized water using Cocarboxylase.)

E. 0.72 mM Coenzyme A Solution (CoA)
   (Prepare 10 ml in deionized water using Coenzyme A, Sodium Salt.)

F. 20 mM \( \beta \)-Nicotinamide Adenine Dinucleotide, Oxidized Form (\( \beta \)-NAD)
   (Prepare 10 ml in deionized water using \( \beta \)-Nicotinamide Adenine Dinucleotide.)
Enzymatic Assay of α-KETOGLUTARATE DEHYDROGENASE

REAGENTS: (continued)

G. 15.6 mM L-Cysteine Solution (Cys-HCl)
(Prepare 10 ml in deionized water using L-Cysteine, Hydrochloride, Monohydrate. Adjust to pH 7.4 at 30°C with 1 M NaOH.)

H. 75 mM α-Ketoglutaric Acid Solution (α-KGA)
(Prepare 10 ml in deionized water using α-Ketoglutaric Acid, Monosodium Salt.)

I. 50 mM MOPS HCl Buffer, pH 7.4 at 30°C (Enz Dil)
(Prepare 100 ml in deionized water using MOPS, Sodium Salt. Adjust to pH 7.4 at 30°C with 1 M HCl.)

J. α-Ketoglutarate Dehydrogenase Enzyme Solution (α-KGDH)
(Immediately before use, prepare a solution containing 0.5 – 1.0 units/ml of α-Ketoglutarate Dehydrogenase in cold Reagent I.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent B (MgCl₂)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent C (CaCl₂)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (Cocarboxylase)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (Coenzyme A)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent F (β-NAD)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent G (Cys-HCl)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent I (α-KGDH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the A₃₄₀nm until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (α-KGA)</td>
<td>0.20</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A₃₄₀nm for approximately 5 minutes. Obtain the rate A₃₄₀nm/minute using the maximum linear rate for both the Test and Blank.
Enzymatic Assay of α-KETOGLUTARATE DEHYDROGENASE

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}})(3)(df)}{(6.22)(0.05)}
\]

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.05 = Volume (in milliliter) of enzyme used

UNIT DEFINITION:

One unit will convert 1.0 µmole of β-NAD to β-NADH per minute at pH 7.4 at 30°C in the presence of saturating levels of coenzyme A.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50.8 mM MOPS, 0.2 mM MgCl₂, 0.01 mM CaCl₂, 0.3 mM cocarboxylase, 0.12 mM coenzyme A, 2.0 mM β-nicotinamide adenine dinucleotide, 2.6 mM L-cysteine, 5.0 mM a-ketoglutaric acid, and 0.025 - 0.05 units a-ketoglutarate dehydrogenase.

NOTES:

1. Cocarboxylase is also known as thiamine pyrophosphate.

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.