Enzymatic Assay of β-HYDROXYACYL-COA DEHYDROGENASE
(EC 1.1.1.35)

PRINCIPLE:

S-Acetoacetyl-CoA + β-NADH \( \text{HOADH} \) > β-Hydroxybutyryl-CoA + β-NAD

Abbreviations used:
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
HOADH = β-Hydroxyacyl-CoA Dehydrogenase
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
CoA = Coenzyme A

CONDITIONS:  T = 37°C, pH = 7.3, \( A_{340 \text{nm}} \), Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.3 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.3 at 37°C with 1 M KOH.)

B. 5.4 mM S-Acetoacetyl Coenzyme A Solution (SAAC)
(Prepare 1 ml in Reagent A using S-Acetoacetyl Coenzyme A, Sodium Salt, Sigma Prod. No. A-1625. Store on ice.)

C. 6.4 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form (β-NADH)
(Prepare 1 ml in cold Reagent A using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. PREPARE FRESH.)

D. β-Hydroxyacyl-CoA Dehydrogenase Enzyme Solution
(Immeditely before use, prepare a solution containing 0.2 – 0.7 unit/ml of β-Hydroxyacyl-CoA Dehydrogenase in cold Reagent A.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent B (SAAC)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent C (β-NADH)</td>
<td>0.05</td>
<td>0.05</td>
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</table>

Mix by inversion and equilibrate to 37°C. Monitor the A$_{340}$nm until constant, using a suitably thermostatted spectrophotometer. Then add:

<p>| | | |</p>
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<thead>
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<tbody>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A$_{340}$nm for approximately 5 minutes. Obtain the $\frac{rA_{340}}{\text{minute}}$ for both the Test and Blank.

CALCULATIONS:

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

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

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of acetoacetyl-CoA to β-hydroxybutyryl-CoA per minute at pH 7.3 at 37°C in the presence of β-NADH.
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 97 mM potassium phosphate, 0.09 mM S-acetoacetyl-coenzyme A, 0.1 mM β-nicotinamide adenine dinucleotide, reduced form and 0.02 - 0.07 unit β-hydroxyacyl-CoA-dehydrogenase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

2. 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.