SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of ACYL COENZYME A SYNTHETASE (EC 6.2.1.3)

PRINCIPLE:

CoA + Oleate + ATP → Oleoyl-CoA + AMP + PP_{i}

ATP + AMP → ADP

2 ADP + 2 Phospho(enol)pyruvate → 2 ATP + 2 Pyruvate

2 Pyruvate + 2 β-NADH → 2 L-Lactate + 2 β-NAD

Abbreviations used:

ATP = Adenosine 5'-Triphosphate
AMP = Adenosine 5'-Monophosphate
ADP = Adenosine 5'-Diphosphate
Oleoyl-CoA = Oleoyl Coenzyme A
PP_{i} = Inorganic Pyrophosphate
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
CoA = Coenzyme A
MK = Myokinase
LDH = L-Lactic Dehydrogenase

CONDITIONS: T = 25°C, pH = 8.1, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Tris Buffer with 20 mM Magnesium Chloride, 2 mM Ethylenediaminetetraacetic Acid (EDTA) and 0.25% (w/v) Triton X-100, pH 8.1 at 25°C

(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, Ethylenediaminetetraacetic Acid, Disodium Dihydrate, Sigma Stock No. ED2SS, and Triton X-100, Sigma Stock No. X-100. Adjust to pH 8.1 at 25°C with 1 M HCl.)
Enzymatic Assay of ACYL COENZYME A SYNTHETASE
(EC 6.2.1.3)

REAGENTS: (continued)

B. 100 mM Tris Solution, pH 7.5 at 25°C
   (Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 25°C with 1 M HCl.)

C. 14.5 mM Adenosine 5'-Triphosphate Solution (ATP)
   (Prepare 1 ml in Reagent B using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. PREPARE FRESH.)

D. 42.7 mM Phospho(enol)pyruvate Solution (PEP)
   (Prepare 1 ml in Reagent B using Phospho(enol)Pyruvate, Trisodium Salt, Hydrate, Sigma Prod. No. P-7002. PREPARE FRESH.)

E. Myokinase Enzyme Solution (MK)
   (Immediately before use, prepare a solution containing 72 units/ml in cold Reagent B using Myokinase², Sigma Prod. No. M-3003.)

F. PK/LDH Mixed Enzymes³ (PK/LDH)
   (Immediately before use, prepare a solution containing 120 units/ml of Pyruvate Kinase in Reagent B using PK²/LDH² Enzymes Suspension, Sigma Stock No. 40-7.)

G. 49 mM Coenzyme A (CoA)
   (Prepare 1 ml in Reagent B using Coenzyme A, Sodium Salt, Sigma Prod. No. C-3144. PREPARE FRESH.)

H. 5.3 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, Sigma Stock No. 340-110, in the appropriate volume of deionized water or prepare 5 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. PREPARE FRESH.)

I. 0.25% (v/v) Triton X-100
   (Prepare 20 ml in deionized water using Triton X-100, Sigma Stock No. X-100.)

J. 0.98 mM Sodium Oleate Solution (Oleate)
   (Prepare 10 ml in Reagent I using Oleic Acid, Sodium Salt, Sigma Prod. No. 0-7501. PREPARE FRESH.)
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REAGENTS: (continued)

K.  50 mM Tris Solution, pH 7.5 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.5 at 25°C with 1 M HCl.)

L.  Acyl Coenzyme A Synthetase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.25 unit/ml of Acyl Coenzyme A Synthetase in cold Reagent K.)

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>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent C (ATP)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (PEP)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (Mk)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent F (PK/LDH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent G (CoA)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent H (β-NADH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} 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 L (Enzyme Solution)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Mix by inversion and monitor the A_{340nm} 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 K (Enzyme Diluent)</td>
<td>------</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent J (Oleate)</td>
<td>0.20</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 15 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.
Enzymatic Assay of ACYL COENZYME A SYNTHETASE
(EC 6.2.1.3)

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(2.8)(df)}{(2)(6.22)(0.2)}
\]

2.8 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.2 = Volume (in milliliter) 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 form 1.0 μmole of AMP and oleoyl-CoA from ATP and oleate per minute at pH 8.1 at 25°C, in the presence of CoA.

FINAL ASSAY CONCENTRATIONS:

In a 2.80 ml reaction mix, the final concentrations are 157 mM Tris, 14 mM magnesium chloride, 1.4 mM ethylenediaminetetraacetic acid, 0.18% (v/v) Triton X-100, 0.26 mM adenosine 5’-triphosphate, 0.76 mM phospho(enol)pyruvate, 4 units myokinase, 6 units pyruvate kinase, 9 units lactic dehydrogenase, 1.8 mM coenzyme A, 0.19 mM β-nicotinamide adenine dinucleotide, reduced form, 0.07 mM oleate and 0.01 - 0.05 unit acyl coenzyme A synthetase.

REFERENCES:

Enzymatic Assay of ACYL COENZYME A SYNTHETASE
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NOTES:

1. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

2. Myokinase Unit Definition: One unit will convert 2.0 µmoles of ADP to ATP and AMP per minute at pH 7.6 at 37°C.

3. Contains not less than 700 units/ml of Pyruvate Kinase and 1000 units/ml of Lactic Dehydrogenase.

4. Pyruvate Kinase Unit Definition: One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.

5. L-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.

6. This assay is based on the cited reference.

7. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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