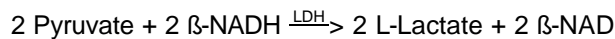
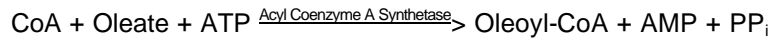


**Enzymatic Assay of ACYL COENZYME A SYNTHETASE
(EC 6.2.1.3)**

PRINCIPLE:



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) Triton¹ 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 Triton¹ 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
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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 PK⁴/LDH⁵ 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) Triton¹ 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.**)

**Enzymatic Assay of ACYL COENZYME A SYNTHETASE
(EC 6.2.1.3)**

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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.00	2.00
Reagent C (ATP)	0.05	0.05
Reagent D (PEP)	0.05	0.05
Reagent E (MK)	0.05	0.05
Reagent F (PK/LDH)	0.05	0.05
Reagent G (CoA)	0.10	0.10
Reagent H (β -NADH)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent L (Enzyme Solution)	0.20	0.20
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Mix by inversion and monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent K (Enzyme Diluent)	-----	0.20
Reagent J (Oleate)	0.20	-----

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 15 minutes. Obtain the $r A_{340nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

**Enzymatic Assay of ACYL COENZYME A SYNTHETASE
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CALCULATIONS:

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

2.8 = Total volume (in milliliters) of assay

df = Dilution factor

2 = 2 moles of β -NAD produced per mole of oleate used

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 μ mole 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:

Shimizu, S., Inoue, K., Tani, Y. and Yamada, H. (1979) *Anal. Biochem.* **98**, 341

**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 μ moles 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 μ mole 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 μ mole 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.

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