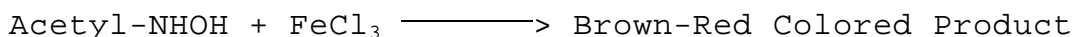
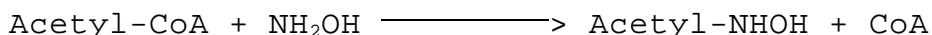
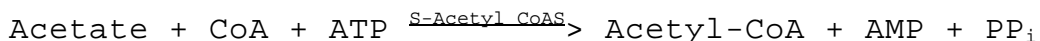


**Enzymatic Assay of S-ACETYL COENZYME A SYNTHETASE
(EC 6.2.1.1)**

PRINCIPLE:



Abbreviations used:

CoA = Coenzyme A

ATP = Adenosine 5'-Triphosphate

S-Acetyl CoAS = S-Acetyl Coenzyme A Synthetase

AMP = Adenosine 5'-Monophosphate

PP_i = Pyrophosphate

Acetyl-CoA = Acetyl Coenzyme A

Acetyl-NHOH = Acethydroxamic Acid

CONDITIONS: T = 37°C, pH = 7.5, A_{546nm}, Light path = 1 cm

METHODS: Colorimetric

REAGENTS:

- A. 500 mM Monobasic Potassium Phosphate (KH₂PO₄)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- B. 500 mM Dibasic Potassium Phosphate (K₂HPO₄)
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)
- C. 500 mM Potassium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 100 ml using Reagent B (K₂HPO₄).
Adjust to pH 7.5 at 37°C with Reagent A (KH₂PO₄).)
- D. 100 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 100 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

**Enzymatic Assay of S-ACETYL COENZYME A SYNTHETASE
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REAGENTS: (continued)

- E. 100 mM Adenosine Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- F. 500 mM Potassium Fluoride Solution (KF)
(Prepare 10 ml in deionized water using Potassium Fluoride, Dihydrate, Sigma Prod. No. P-6642.)
- G. 50 mM Potassium Acetate Solution (KOAC)
(Prepare 10 ml in deionized water using Potassium Acetate, Sigma Prod. No. P-1147.)
- H. 100 mM Reduced Glutathione Solution (GSH)
(Prepare 10 ml in deionized water using Glutathione, Reduced Form, Free Acid, Sigma Prod. No. G-4251.)
- I. 7.8 mM Coenzyme A Solution (CoA)
(Prepare 10 ml in deionized water using Coenzyme A, Sodium Salt, Sigma Prod. No. C-3144.)
- J. 1000 mM Hydroxylamine Solution (HA)
(Prepare 20 ml in deionized water using Hydroxylamine, Hydrochloride, Sigma Prod. No. H-9876.)
- K. 370 mM Iron Chloride and 3.3% (w/v) Trichloroacetic Acid Solution (FeCl₃)
(Prepare 100 ml by dissolving 10 g of Ferric Chloride, Hexahydrate, Prod. No. F-2877 and 3.3 g of Trichloroacetic Acid, Sigma Prod. No. T-4885 in 80 ml of deionized water. Add 6.6 ml of Hydrochloric Acid, Sigma Prod. No. H-7020 and make up to 100 ml with deionized water.)
- L. S-Acetyl Coenzyme A Synthetase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of S-Acetyl Coenzyme A Synthetase in cold Reagent C.)

PROCEDURE:

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Reagent C (Buffer) | 0.20 | 0.20 |

Reagent D (MgCl_2)

0.05

0.05

**Enzymatic Assay of S-ACETYL COENZYME A SYNTHETASE
(EC 6.2.1.1)**

PROCEDURE: (continued)

| | Test | Blank |
|------------------|-------|-------|
| Reagent E (ATP) | 0.10 | 0.10 |
| Reagent F (KF) | 0.10 | 0.10 |
| Reagent G (KOAC) | 0.20 | 0.20 |
| Reagent H (GSH) | 0.10 | 0.10 |
| Reagent I (CoA) | 0.05 | ----- |
| Reagent J (HA) | 0.20 | 0.20 |
| Deionized Water | ----- | 0.05 |

Mix by inversion and equilibrate to 37°C. Then add:

| | | |
|-----------------------------|------|------|
| Reagent L (Enzyme Solution) | 0.10 | 0.10 |
|-----------------------------|------|------|

Immediately mix by inversion and incubate for 20 minutes at 37°C. Then add:

| | | |
|--------------------------------|------|------|
| Reagent K (FeCl ₃) | 2.00 | 2.00 |
|--------------------------------|------|------|

Mix by inversion and transfer to suitable cuvettes. Record the A_{546nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(A_{546\text{nm}} \text{ Test} - A_{546\text{nm}} \text{ Blank})(3.1)}{(20)(0.98)(\text{mg enzyme/RM})}$$

3.1 = Total Volume (in milliliters) of assay

0.98 = Change in absorbance of the formation of one μmole of

the brown-red colored product

20 = Time of assay (in minutes) of acethydroxamic acid under

the conditions of the assay.

RM = Reaction Mix

UNIT DEFINITION:

One unit will form 1.0 μmole of S-Acetyl Coenzyme A from acetate, ATP and coenzyme A per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 136 mM potassium phosphate, 4 mM magnesium chloride, 9.1 mM ATP, 45 mM potassium fluoride, 9.1 mM potassium acetate, 9.1 mM reduced glutathione, 0.35 mM coenzyme A, 182 mM hydroxylamine and 0.02-0.04 unit S-acetyl coenzyme

A synthetase.

**Enzymatic Assay of S-ACETYL COENZYME A SYNTHETASE
(EC 6.2.1.1)**

REFERENCES:

Berg, P. (1956) *Journal of Biological Chemistry*, **222**, 991-1013.

NOTES:

1. This assay is a modification of the enzyme assay described in the cited references.
2. All products 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.