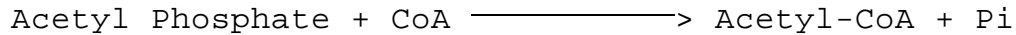


**Determination of the Concentration and Molecular Weight
of COENZYME A using PHOSPHOTRANSACETYLASE**

PRINCIPLE:



Abbreviations used:

CoA = Coenzyme A

Acetyl-CoA = Acetyl Coenzyme A

Pi = Inorganic Phosphate

CONDITIONS: T = 25°C, pH = 7.6, $A_{233\text{nm}}$, Light path = 1 cm

METHODS: Spectrophotometric

REAGENTS:

- A. 100 mM Tris Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.6 at 25°C with 1 M HCl.)
- B. 100 mM Acetyl Phosphate Solution
(Prepare 10 ml in deionized water using Acetyl Phosphate, Lithium Potassium Salt, Prod. No. A-0262. **PREPARE FRESH.**)
- C. Coenzyme A Solution (CoA)
(Weigh two samples accurately, approximately 3.0 mg and dissolve each in 10 ml of deionized water which has been purged with nitrogen.)
- D. Phosphotransacetylase Enzyme Solution
(Prepare a solution containing 100 units/ml of Phosphotransacetylase, from *Clostridium kluveri*, Prod. No. P-5907, in Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.90	1.90
Reagent B (Acetyl Phosphate)	0.10	-----
Reagent C (CoA)	1.00	1.00
Deionized Water	-----	0.10

Mix by inversion and equilibrate to 25°C. Monitor the $A_{233\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.02	0.02
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Mix by inversion and allow the reaction to proceed for 10 minutes. Upon completion of the reaction record the $A_{233\text{nm}}$ and calculate the $\Delta A_{233\text{nm}}$ and the apparent molecular weight.

CALCULATIONS:

$$\Delta A = A_i - A_f \quad \begin{array}{l} A_i = \text{Initial absorbance} \\ A_f = \text{Final absorbance} \end{array}$$

$$\text{Micromoles CoA/weighed sample} = \frac{\Delta A (3.02) (10)}{4.44}$$

3.02 = Volume of assay

10 = Sample dilution factor

4.44 = Extinction coefficient of acetyl coenzyme A at 233 nm

$$\text{apparent mw} = \frac{\text{mg sample weighed} \times 1000}{\mu\text{moles CoA/weighed sample}}$$

FINAL CONCENTRATION:

In a 3.02 ml reaction mix, the final concentrations are 63.6 mM tris HCl, 3.3 mM acetyl phosphate, and 2.0 unit phosphotransacetylase.

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REFERENCES:

(1974) *Methods of Enzymatic Analysis*, pp. 1972

NOTES:

1. 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.