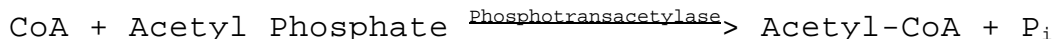


**Enzymatic Assay of PHOSPHOTRANSACETYLASE
(EC 2.3.1.8)**

PRINCIPLE:



Abbreviations used:

CoA = Coenzyme A

Acetyl-CoA = Acetyl Coenzyme A

P_i = Inorganic phosphate

CONDITIONS: T = 25°C, pH = 7.4, A_{233nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 7.4 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.4 at 25°C with 5 M HCl.)
- B. 100 mM Glutathione Solution (Gluth)
(Prepare 10 ml in Reagent A using Glutathione, Free Acid, Reduced Form, Sigma Prod. No. G-4251. **PREPARE FRESH.**)
- C. 6.5 mM Coenzyme A Solution (CoA)
(Prepare 2 ml in Reagent A using Coenzyme A, Sodium Salt, Sigma Prod. No. C-3019. **PREPARE FRESH.**)
- D. 220 mM Acetyl Phosphate Solution (Acet Phos)
(Prepare 1 ml in Reagent A using Acetyl Phosphate, Lithium Potassium Salt, Sigma Prod. No. A-0262. **PREPARE FRESH.**)
- E. 1 M Ammonium Sulfate Solution ((NH₄)₂SO₄)
(Prepare 50 ml in deionized water using Ammonium Sulfate, Sigma Prod. No. A-5132.)
- F. 25 mM Tris HCl Buffer with 500 mM Ammonium Sulfate, pH 8.0 at 25°C (Enzyme Diluent)
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503 and Ammonium Sulfate, Sigma Prod. No. A-5132. Adjust to pH 8.0 at 25°C with 1 M

HCl.)

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REAGENTS: (continued)

G. Phosphotransacetylase Enzyme Solution
(Immediately before use, prepare a solution containing
1 - 2 units of phosphotransacetylase in cold
Reagent F.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.60	2.60
Reagent B (Gluth)	0.05	0.05
Reagent C (CoA)	0.20	0.20
Reagent D (Acet Phos)	0.10	0.10
Reagent E ((NH ₄) ₂ SO ₄)	0.03	0.03

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

Reagent G (Enzyme Solution)	0.02	-----
Reagent F (Enzyme diluent)	-----	0.02

Immediately mix by inversion and record the increase in
A_{233nm} for approximately 5 minutes. Obtain the r A_{233nm}/minute
using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(\bar{r} A_{233\text{nm}}/\text{min Test} - \bar{r} A_{233\text{nm}}/\text{min Blank})(3)(\text{df})}{(4.44)(0.02)}$$

3.0 = Total volume (in milliliters) of assay

df = Dilution factor

4.44 = Millimolar extinction coefficient of Acetyl-CoA at
233 nm

0.02 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will convert 1.0 μ mole of CoA to acetyl-CoA per minute at pH 7.4 at 25°C using acetyl phosphate as substrate.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 98.5 mM Tris, 1.6 mM glutathione, 0.43 mM coenzyme A, 7.23 mM acetyl phosphate, 13.3 mM ammonium sulfate and 0.02 - 0.04 unit phosphotransacetylase.

REFERENCE:

Klotzsch, H.R. (1969) *Methods in Enzymology*, XIII, 381-386

NOTES:

1. 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.