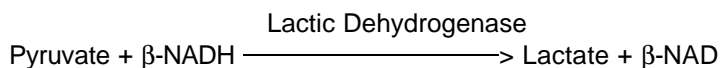
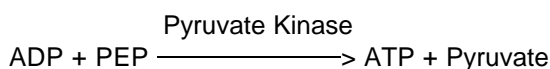
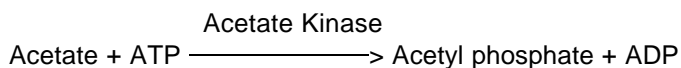


**Enzymatic Assay of ACETATE KINASE
(EC 2.7.2.1)
from Bacillus stearothermophilus**

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

PEP = Phospho(enol)Pyruvate

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 30°C, pH = 7.2, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Imidazole Solution
(Prepare 50 ml in deionized water using Imidazole, Sigma Prod. No. I-0250.)
- B. Acetic Acid, Glacial (HOAc)
(Use Acetic Acid, Glacial, Sigma Prod. No. A-6283.)
- C. 102 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 5 ml in deionized water using Phospho(enol)pyruvate, Monopotassium Salt, Sigma Prod. No. P-7127. **PREPARE FRESH.**)
- D. 300 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 3 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. **PREPARE FRESH.**)

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REAGENTS: (continued)

- E. 7.8 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, Stock No. 340-110, in the appropriate volume of deionized water or dissolve β -Nicotinamide Adenine Dinucleotide, Reduced Form, Sigma Prod. No. N-8129, in the appropriate volume of water.)
- F. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Sterile filtered Solution, Sigma Prod. No. M-1028.)
- G. PK/LDH Enzymes Solution¹
(Use PK/LDH Enzymes Solution in 50% Glycerol, Sigma Prod. No. P-0294.)
- H. Acetate Kinase Enzyme Solution
(Immediately before use, prepare a solution containing 0.50 - 0.75 unit/ml of Acetate Kinase in cold deionized water.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

| | |
|------------------------|-------|
| Reagent A (Imidazole) | 18.20 |
| Reagent B (HOAc) | 0.74 |
| Reagent C (PEP) | 1.00 |
| Reagent D (ATP) | 1.00 |
| Reagent F ($MgCl_2$) | 2.00 |

Mix by swirling and adjust to pH 7.2 at 30°C with 1 M KOH. Then add:

| | |
|----------------------------|------|
| Reagent E (β -NADH) | 1.00 |
|----------------------------|------|

Mix by swirling. Then add deionized water to make a final volume of 30 ml. Mix by stirring.

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Reaction Cocktail | 2.80 | 2.80 |
| Reagent G (PK/LDH) | 0.10 | 0.10 |

**Enzymatic Assay of ACETATE KINASE
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | | |
|-----------------------------|-------|-------|
| Deionized Water | ----- | 0.10 |
| Reagent H (Enzyme Solution) | 0.10 | ----- |

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 10 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

0.1 = 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 phosphorylate 1.0 μmole of acetate to acetyl phosphate per minute at pH 7.2 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 57 mM imidazole, 400 mM acetic acid, 3.2 mM phospho(enol)-pyruvate, 9.3 mM adenosine 5'-triphosphate, 19 mM magnesium chloride, 0.24 mM β -nicotinamide adenine dinucleotide, 70 units pyruvate kinase, 100 units lactic dehydrogenase, and 0.05 - 0.075 unit acetate kinase.

**Enzymatic Assay of ACETATE KINASE
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from *Bacillus stearothermophilus***

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume 1, 425-426, Academic Press, Inc., New York, NY

NOTES:

1. Contains approximately than 700 units/ml of Pyruvate Kinase and 1000 units/ml of Lactic Dehydrogenase.
2. 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.
3. 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.
4. This assay is based on the cited reference.
5. 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.