

**Enzymatic Assay of ACETATE KINASE<sup>1</sup>**  
**(EC 2.7.2.1)**

**PRINCIPLE:**

Acetate + ATP Acetate Kinase > Acetyl Phosphate + ADP

ADP + PEP Pyruvate Kinase > ATP + Pyruvate

Pyruvate +  $\beta$ -NADH Lactic Dehydrogenase > Lactate +  $\beta$ -NAD

Abbreviations used:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

PEP = Phospho(enol)Pyruvate

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 25°C, pH = 7.6, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 100 mM Triethanolamine Buffer, pH 7.6 at 25°C.  
(Prepare 50 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. 1 M Sodium Acetate Solution (NaOAc)  
(Prepare 10 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. **PREPARE FRESH.**)
- C. 91 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)

- D. 56 mM Phospho(enol)pyruvate Solution (PEP)  
(Prepare 1.5 ml in deionized water using Phospho(enol)pyruvate, Mono(cyclohexylammonium) Salt, Sigma Prod. No. P-3637. **PREPARE FRESH.**)
- E. 200 mM Magnesium Chloride Solution (MgCl<sub>2</sub>)  
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- F. 6.4 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)  
(Dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- G. PK/LDH Enzymes Suspension<sup>2</sup>  
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)
- H. Myokinase Enzyme Solution (MK)  
(Immediately before use, prepare a solution containing 2000 - 3000 units/ml in cold deionized water using Myokinase, Sigma Prod. No. M-3003.)
- I. Acetate Kinase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.2 - 0.5 unit/ml of Acetate Kinase in cold Reagent A.)

**PROCEDURE:**

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

Reagent A (Buffer)	17.80
Reagent B (NaOAc)	6.00
Reagent E (MgCl <sub>2</sub> )	1.00
Reagent F (β-NADH)	0.50

Mix and adjust to pH 7.6 at 25°C with 0.1 M HCl or 0.1 M NaOH, if necessary.

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**PROCEDURE:** (continued)

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

	Test	Blank
Reaction Cocktail	2.53	2.53
Reagent G (PK/LDH)	0.05	0.05
Reagent H (MK)	0.02	0.02

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

Reagent C (ATP)	0.20	0.20
Reagent D (PEP)	0.10	0.10
Reagent A (Buffer)	-----	0.10
Reagent I (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the decrease in  $A_{340\text{nm}}$  for approximately 5 minutes. Obtain the  $\Delta A_{340\text{nm}}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}})(3.0)(df)}{(6.22)(0.1)}$$

3.0 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu\text{mole}$  of acetate to acetyl phosphate per minute at pH 7.6 at 25°C.

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**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 63 mM triethanolamine, 200 mM sodium acetate, 6.1 mM adenosine 5'-triphosphate, 1.9 mM phospho(enol)pyruvate, 6.7 mM magnesium chloride, 0.11 mM  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 35 units pyruvate kinase, 50 units lactic dehydrogenase, 40 - 60 units myokinase, and 0.02 - 0.05 unit acetate kinase.

**REFERENCES:**

Bergmeyer, I.U. (1983) *Methods of Enzymatic Analysis*, 3rd ed., II, 127-128

Rose, I.A., Grunberg-Manago, M., Korey, S.R., and Ochoa, S. (1954) *Journal of Biological Chemistry* **211**, 737-756

**NOTES:**

1. Assay not to be used for Acetate Kinase from *Bacillus stearothermophilus*, Sigma Prod. No. A-6781.
2. Contains not less than 700 Pyruvate Kinase units and 1000 Lactic Dehydrogenase units per ml.
3. Pyruvate Kinase Unit Definition: One unit will convert 1.0  $\mu$ mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
4. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0  $\mu$ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
5. Myokinase Unit Definition: One unit will convert 2.0  $\mu$ moles of ADP to ATP and AMP per minute at pH 7.6 at 37°C.
6. This assay is based on the cited references.
7. 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.**