

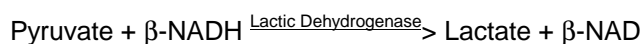
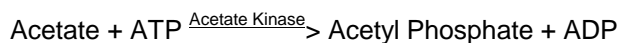


SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of ACETATE KINASE¹ (EC 2.7.2.1)

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 = 25°C, pH = 7.6, A_{340nm}, 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.**)

Enzymatic Assay of ACETATE KINASE¹ (EC 2.7.2.1)

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 (MgCl₂)
(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 Suspension²
(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 ₂) | 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.

Enzymatic Assay of ACETATE KINASE¹ (EC 2.7.2.1)

PROCEDURE: (continued)

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| 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 $\Delta A_{340\text{nm}}$ 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:

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

3.0 = 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 7.6 at 25°C.

Enzymatic Assay of ACETATE KINASE¹ (EC 2.7.2.1)

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 β -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 μ 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 μ 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 μ 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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.