SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of ACETATE KINASE[1] (EC 2.7.2.1)

PRINCIPLE:

Acetate + ATP $\xrightarrow{\text{Acetate Kinase}}$ Acetyl Phosphate + ADP

ADP + PEP $\xrightarrow{\text{Pyruvate Kinase}}$ ATP + Pyruvate

Pyruvate + β-NADH $\xrightarrow{\text{Lactic Dehydrogenase}}$ Lactate + β-NAD

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.**)

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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 ($MgCl_2$)
(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[2]
(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.

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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 ΔA_{340nm} 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_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3.0 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of 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.

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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 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 μmole 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 μmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

5. Myokinase Unit Definition: One unit will convert 2.0 μmoles 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.