SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of PYRUVATE KINASE
(EC 2.7.1.40)
Sigma Prod. No. P-1903

PRINCIPLE:

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

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

Abbreviations used:
ADP = Adenosine 5'-Diphosphate
PEP = Phospho(enol)Pyruvate
ATP = Adenosine 5'-Triphosphate
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Imidazole HCl Buffer, pH 7.2 at 30°C.
(Prepare 50 ml in deionized water using Imidazole, Sigma Prod. No. I-0250. Adjust to pH 7.2 with 1 M HCl.)

B. 100 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 10 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-2754. **PREPARE FRESH.**)

C. 1000 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

D. 2500 mM Potassium Chloride Solution (KCl)
(Prepare 10 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)

REAGENTS: (continued)

E. 155 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 10 ml in deionized water using Phospho(enol)pyruvate, Mono(Cyclohexylammonium) Salt, Sigma Prod. No. P-3637. **PREPARE FRESH.**)

F. 13.1 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** prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH.**)

G. L-Lactic Dehydrogenase Enzyme Solution (LDH)
(Immediately before use, prepare a solution containing 400 units/ml in deionized water using L-Lactic Dehydrogenase, Sigma Prod. No. L-2500.)

H. Pyruvate Kinase Enzyme Solution (PK)
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of Pyruvate Kinase in cold deionized water.)

PROCEDURE:

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

Reagent A (Buffer)	19.00
Reagent B (ADP)	2.00
Reagent C ($MgCl_2$)	0.40
Reagent D (KCl)	0.75
Deionized water	2.50
Reagent F (β-NADH)	0.38

Mix and adjust to pH 7.2 at 30°C with 100 mM HCl or 100 mM KOH, if necessary.

PROCEDURE: (continued)

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

	Test	Blank
Reaction Cocktail	2.75	2.85
Reagent G (LDH)	0.05	0.05
Reagent E (PEP)	0.10	------

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

Deionized Water	------	0.10
Reagent H (PK)	0.10	------

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

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340nm
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.2 at 30°C.

Enzymatic Assay of PYRUVATE KINASE
(EC 2.7.1.40)
Sigma Prod. No. P-1903

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 72 mM imidazole, 7.6 mM adenosine 5'-diphosphate, 15.2 mM magnesium chloride, 71.2 mM potassium chloride, 5.2 mM phospho(enol)pyruvate, 0.19 mM β-nicotinamide adenine dinucleotide, reduced form, 20 units L-lactic dehydrogenase and 0.015 - 0.030 unit pyruvate kinase.

NOTES:

1. L-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.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.