Enzymatic Assay of PYRUVATE KINASE
(EC 2.7.1.40)

PRINCIPLE:

Phospho(enol)pyruvate + ADP $\xrightarrow[Mg^{2+},\ K^{+}]{\text{Pyruvate Kinase}}$ Pyruvate + ATP

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

Abbreviations used:
ADP = Adenosine 5'-Diphosphate
ATP = Adenosine 5'-Triphosphate
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 37°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.6 at 37°C.
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.6 at 37°C with 1 M KOH.)

B. 17 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 1 ml in deionized water using Phospho(enol)pyruvate, Trisodium Salt, Hydrate, Sigma Prod. No. P-7002. **PREPARE FRESH**.)

C. 1.3 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of a 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt,
Sigma Prod. No. N-8129, in the appropriate volume of Reagent A. **PREPARE FRESH.**)

D. 100 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 1 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

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REAGENTS: (continued)

E. 44 mM Adenosine Diphosphate Solution (ADP)
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-8146.)

F. L-Lactic Dehydrogenase Solution (LDH)
(Immediately before use, prepare a solution containing 5000 units/ml in cold Reagent A using L-Lactic Dehydrogenase, Sigma Prod. No. L-2500.)

G. Pyruvate Kinase
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of Pyruvate Kinase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Deionized Water	1.40	1.40
Reagent A (Buffer)	0.80	0.90
Reagent B (PEP)	0.10	0.10
Reagent C (ß-NADH)	0.25	0.25
Reagent D ($MgSO_4$)	0.20	0.20
Reagent E (ADP)	0.10	0.10
Reagent F (LDH)	0.002	0.002

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (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.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{340nm}\text{/min Test} - \text{r } A_{340nm}\text{/min Blank})(2.952)(df)}{(6.22)\ (0.1)}$$

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

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.952 ml reaction mix, the final concentrations are 39 mM potassium phosphate, 0.58 mM phospho(enol)pyruvate, 0.11 mM ß-nicotinamide adenine dinucleotide, reduced form, 6.8 mM magnesium sulfate, 1.5 mM adenosine 5'-diphosphate, 10 units lactic dehydrogenase and 0.015 - 0.030 unit pyruvate kinase.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Second Edition, Volume I, 509-510, Academic Press, Inc., New York, NY

NOTES:

1. 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.

NOTES: (continued)

2. This assay is based on the cited reference.

3. 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.