Enzymatic Assay of PYRUVATE KINASE
(EC 2.7.1.40)
From Rabbit Liver

PRINCIPLE:

$$\text{Phospho(enol)pyruvate} + \text{ADP} \xrightarrow[Mg2^{+}]{\text{Pyruvate Kinase}} \text{Pyruvate} + \text{ATP}$$

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

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. 8.0 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 1 ml in deionized water using Phospho(enol)Pyruvate, Monopotassium Salt, Sigma Prod. No. P-7127.)

C. 3 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Dissolve the contents of a 10 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of Reagent A. **PREPARE FRESH.**)

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

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REAGENTS: (continued)

E. 40 mM Adenosine Diphosphate Solution (ADP)
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Di(Monocyclohexylammonium) Salt, Sigma Prod. No. A-4386.)

F. L-Lactic Dehydrogenase Solution (LDH)
(Immediately before use, prepare a solution containing 500 units/ml of L-Lactic Dehydrogenase, Sigma Prod. No. L-2500, in cold Reagent A.)

G. 30 mM Fructose 1,6-Diphosphate Solution (F 1,6-P)
(Prepare 2 ml in deionized water using Fructose 1,6-Diphosphate, Sodium Salt, Sigma Prod. No. F-4757.)

H. Pyruvate Kinase
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Pyruvate Kinase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Deionized Water	1.30	1.30
Reagent A (Buffer)	0.80	0.90
Reagent B (PEP)	0.16	0.16
Reagent C (ß-NADH)	0.20	0.20
Reagent D (Magnesium Sulfate)	0.20	0.20
Reagent E (ADP)	0.10	0.10
Reagent F (LDH)	0.04	0.04
Reagent G (F 1,6-P)	0.10	0.10

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

Reagent H (Enzyme Solution)	0.10	------

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

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CALCULATIONS:

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

3 = 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

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C in the presence of
1.0 mM of fructose 1,6-diphosphate. In the absence of added fructose 1,6-diphosphate which acts as an (activator), considerably lower activity will be observed.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 38 mM potassium phosphate, 0.43 mM phospho(enol)pyruvate, 0.2 mM ß-nicotinamide adenine dinucleotide, 6.7 mM magnesium sulfate, 1.3 mM adenosine 5'-diphosphate, 20 units lactic dehydrogenase, 1 mM fructose 1,6-diphosphate and
0.03 to 0.06 unit pyruvate kinase.

REFERENCE:

Cardenas, J.M. and Dyson, R.D. (1973) *Journal of Biological Chemistry* **248**, 6938-6944.

NOTES:

1. Lactic Dehydrogenase Unit Definition: One unit will

reduce 1.0 micromole 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.