

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

PRINCIPLE:

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

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

Abbreviations used:

ADP = Adenosine 5'-Diphosphate

ATP = Adenosine 5'-Triphosphate

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -NAD = β -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 β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of a 10 mg vial of β -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:

	<u>Test</u>	<u>Blank</u>
Deionized Water	1.30	1.30
Reagent A (Buffer)	0.80	0.90
Reagent B (PEP)	0.16	0.16
Reagent C (β -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 $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r_{A_{340\text{nm}}}$ /minute using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -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 μ mole 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 β -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.

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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.