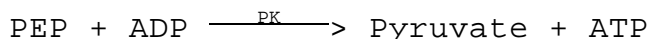


**Determination of the Concentration and
Molecular Weight of
PHOSPHO(ENOL)PYRUVATE**

PRINCIPLE:



Abbreviations:

PEP = Phospho(enol)pyruvate

ADP = Adenosine-5'-Diphosphate

ATP = Adenosine-5'-Triphosphate

PK = Pyruvate Kinase

LDH = Lactate Dehydrogenase

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: Spectrophotometric

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.6 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 7.6 at 37°C with 1 M KOH.)
- B. Phospho(enol)pyruvate Solution (PEP)
(Weigh approximately 1.5 mg of Phospho(enol)pyruvate and dissolve in 10 ml of deionized water. Dilute to 0.06 mg/ml in deionized water.)
- C. 2.5 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Dissolve the contents of one vial of β -Nicotinamide Adenine Dinucleotide, Reduced Form Disodium, Stock No. 340-105 in 2.5 ml of cold Reagent A.)

**Determination of the Concentration and
Molecular Weight of
PHOSPHO(ENOL)PYRUVATE**

REAGENTS: (continued)

- D. 1000 mM Potassium Chloride Solution (KCl)
(Prepare 5 ml in deionized water using Potassium Chloride, Prod. No. P-4504.)
- E. 50 mM Magnesium Sulfate Solution (MgSO₄)
(Prepare 5 ml in deionized water using Magnesium Sulfate, Heptahydrate, Prod. No. M-1880.)
- F. 40 mM Adenosine-5'-Diphosphate Solution (ADP)
(Prepared 5 ml in deionized water using Adenosine 5'-Diphosphate, Di(Monocyclohexylammonium) Salt, Prod. No. A-4386.)
- G. PK/LDH Mixed Enzymes
(Use PK/LDH Enzymes Suspension, Stock No. 40-7, undiluted.)¹

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.48	2.48
Reagent B (PEP)	1.00	-----
Reagent C (β-NADH)	0.13	0.13
Reagent D (KCl)	0.08	0.08
Reagent E (MgSO ₄)	0.16	0.16
Reagent F (ADP)	0.10	0.10

Mix by inversion and equilibrate for 5 minutes at 37°C and record the initial A_{340nm} using a suitably thermostatted spectrophotometer. Then add:

Reagent G (PK/LDH)	0.05	0.05
--------------------	------	------

Mix by inversion and allow the reaction to proceed for 15 minutes. Record the final A_{340nm} for both the Test and Blank.

**Determination of the Concentration and
Molecular Weight of
PHOSPHO(ENOL)PYRUVATE**

CALCULATIONS:

$$\Delta A = A_i - A_f$$

A_i = Initial absorbance

A_f = Final absorbance

$$\text{micromoles PEP/weighed sample} = \frac{(\Delta A)(3.00)(25)}{(6.22)}$$

3.00 = Total volume of Reaction Mix

25 = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

$$\text{Apparent molecular weight} = \frac{\text{mg sample weighed} \times 1000}{\text{umoles PEP/weighed sample}}$$

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 54 mM potassium phosphate, 0.11 mM β -NADH, 27 mM KCl, 2.7 mM MgSO₄, 1.3 mM ADP, 35 units PK and 50 units LDH.

NOTES:

1. Contains not less than 700 pyruvate kinase units and 1000 lactic dehydrogenase units per ml.
2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.