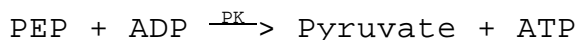


**Determination of the Concentration and Molecular Weight of
ADENOSINE 5'-DIPHOSPHATE**

PRINCIPLE:



Abbreviations used:

PEP = Phospho(enol)pyruvate

ADP = Adenosine 5'-Diphosphate

PK = Pyruvate Kinase

ATP = Adenosine 5'-Triphosphate

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

LDH = Lactate Dehydrogenase

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Determination

REAGENTS:

- A. 150 mM Triethanolamine HCl Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. 32 mM Phospho(enol)pyruvate, 83 mM Magnesium Sulfate, and 135 mM Potassium Chloride Solution (PEP)
(Prepare 2 ml in deionized water using Phospho(enol)pyruvate, Tri(cyclohexylammonium)Salt, Sigma Prod. No. P-7252, Magnesium Sulfate, Sigma Prod. No. M-1880, and Potassium Chloride, Sigma Prod. No. P-4504.)
- C. 3.8 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form (β -NADH)
(Prepare 2 ml in Reagent A using β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)

**Determination of the Concentration and Molecular Weight of
ADENOSINE 5'-DIPHOSPHATE**

REAGENTS: (continued)

- D. Adenosine 5'-Diphosphate Solution (ADP)
(Weigh approximately 2.5 mg of Adenosine 5'-Diphosphate and dissolve in 25 ml of deionized water.)
- E. L-Lactic Dehydrogenase Enzyme Suspension (LDH)¹
(Use L-Lactic Dehydrogenase, Sigma Prod. No. L-2500.)
- F. Pyruvate Kinase Enzyme Suspension (PK)²
(Use Pyruvate Kinase, Sigma Prod. No. P-1506.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.70	1.80
Reagent B (PEP)	0.15	0.15
Reagent C (β-NADH)	0.10	-----
Reagent D (ADP)	1.00	1.00
Reagent E (LDH)	0.01	0.01

Mix by inversion and equilibrate to 25°C using a suitably thermostatted spectrophotometer. Record the initial $A_{340\text{nm}}$ for both the Test and Blank. Then add:

Reagent F (PK)	0.02	0.02
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Immediately mix by inversion and allow the reaction to proceed to completion (approximately 5 minutes). Record the final $A_{340\text{nm}}$ for both the Test and Blank.

CALCULATION:

$$r A = A_i - A_f$$

A_i = Initial Absorbance

A_f = Final Absorbance

$$\text{Micromoles ADP/weighed sample} = \frac{(r A \text{ Test} - r A \text{ Blank})(2.98)(25)}{6.22}$$

2.98 = Total volume (in milliliters) of assay

25 = Dilution factor

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

**Determination of the Concentration and Molecular Weight of
ADENOSINE 5'-DIPHOSPHATE**

CALCULATIONS: (continued)

$$\text{Apparent Molecular Weight} = \frac{\text{mg sample weighed} \times 1000}{\mu\text{moles ADP/weighed sample}}$$

FINAL ASSAY CONCENTRATIONS:

In a 2.98 ml reaction mix, the final concentrations are 91 mM triethanolamine, 1.6 mM phospho(enol)pyruvate, 4.2 mM magnesium sulfate, 6.8 mM potassium chloride, 0.13 mM β -nicotinamide adenine dinucleotide, reduced form, 40 units pyruvate kinase, 100 units lactic dehydrogenase, and varying amounts of adenosine 5'-diphosphate.

REFERENCE:

Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume 4, 2149-2152, Academic Press, Inc, New York, NY

NOTES:

1. Contains not less than 10,000 units of L-lactic dehydrogenase per ml.
2. Contains not less than 2,000 units of pyruvate kinase per ml.
3. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
4. Pyruvate Kinase 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.
5. This assay is based on the cited reference.
6. 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.