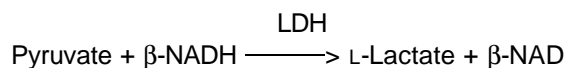
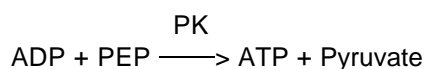
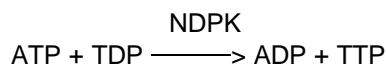


## Enzymatic Assay of NUCLEOSIDE 5'-DIPHOSPHATE KINASE (EC 2.7.4.6)

### PRINCIPLE:



#### Abbreviations:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

TDP = Thymidine 5'-Diphosphate

NDPK = Nucleoside 5'-Diphosphate Kinase

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

PEP = Phospho(enol)pyruvate

LDH = Lactic Dehydrogenase

PK = Pyruvate Kinase

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

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 100 mM Triethanolamine 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. 21 mM Thymidine 5'-Diphosphate Solution (TDP)  
(Prepare 2 ml in deionized water using Thymidine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. T-9375.)
- C. 33 mM Adenosine 5'-Triphosphate Solution (ATP)  
(Prepare 3 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- D. 12 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Prepare by dissolving the contents of one 10 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH.**)

**Enzymatic Assay of NUCLEOSIDE 5'-DIPHOSPHATE KINASE  
(EC 2.7.4.6)**

**REAGENTS:**

- E. 500 mM Magnesium Chloride and 2000 mM Potassium Chloride Solution (MgCl<sub>2</sub>/KCl)  
(Prepare 10 ml in deionized water using Magnesium Chloride, 4.9 M Solution, Sigma Stock No. 104-20 and Potassium Chloride, Sigma Prod. No. P-4504.)
- F. 33 mM Phospho(enol)pyruvate Solution (PEP)  
(Prepare 2 ml in Reagent E using Phospho(enol)pyruvate, Tri(cyclohexylammonium Salt), Sigma Prod. No. P-7252.)
- G. PK/LDH Mixed Enzyme Solution (PK/LDH)  
(Use PK/LDH Enzymes suspension<sup>1</sup>, Sigma Stock No. 40-7.)
- H. Nucleoside 5'-Diphosphate Kinase Enzyme Solution (NDPK)  
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Nucleoside 5'-Diphosphate Kinase in cold deionized water.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.49	2.49
Reagent B (TDP)	0.10	0.10
Reagent C (ATP)	0.20	0.20
Reagent D (β-NADH)	0.05	0.05
Reagent F (PEP)	0.10	0.10
Reagent G (PK/LDH)	0.01	0.01

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (NDPK)	0.05	-----
Deionized Water	-----	0.05

Immediately mix by inversion and record the decrease in A<sub>340nm</sub> for approximately 5 minutes. Obtain the ΔA<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.05)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.05 = Volume (in milliliters) of assay

## Enzymatic Assay of NUCLEOSIDE 5'-DIPHOSPHATE KINASE (EC 2.7.4.6)

### CALCULATIONS: (continued)

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

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

### UNIT DEFINITION:

One unit will convert 1.0  $\mu$ mole each of TDP and ATP to TTP and ADP per minute at pH 7.6 at 25°C in a coupled system with PK/LDH.

### FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 83 mM triethanolamine, 0.70 mM thymidine 5'-diphosphate, 2.2 mM adenosine 5'-triphosphate, 0.2 mM  $\beta$ -NADH, 1.1 mM phospho(enol)pyruvate, 16.7 mM magnesium chloride, 66.7 mM potassium chloride, 10 units lactic dehydrogenase, 7 units pyruvate kinase and 0.025 - 0.050 unit nucleoside 5'-diphosphate kinase.

### REFERENCE:

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, 2nd edition, Volume II, 488-489.

### NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 Lactic Dehydrogenase units per ml.
2. Unit Definition for L-Lactic Dehydrogenase: One unit will reduce 1.0  $\mu$ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
3. Unit Definition for Pyruvate Kinase: One unit will convert 1.0  $\mu$ mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
4. 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.**