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

**PRINCIPLE:**

\[
\begin{align*}
&\text{NDPK} \\
&\text{ATP} + \text{TDP} \rightarrow \text{ADP} + \text{TTP} \\
&\text{PK} \\
&\text{ADP} + \text{PEP} \rightarrow \text{ATP} + \text{Pyruvate} \\
&\text{LDH} \\
&\text{Pyruvate} + \beta-\text{NADH} \rightarrow \text{L-Lactate} + \beta-\text{NAD}
\end{align*}
\]

**Abbreviations:**
- ATP = Adenosine 5'-Triphosphate
- ADP = Adenosine 5'-Diphosphate
- TDP = Thymidine 5'-Diphosphate
- NDPK = Nucleoside 5'-Diphosphate Kinase
- β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
- β-NAD = β-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 β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)  
(Prepare by dissolving the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH**.)
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REAGENTS:

E. 500 mM Magnesium Chloride and 2000 mM Potassium Chloride Solution (MgCl₂/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 suspension¹, 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:

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

Mix by inversion and equilibrate to 25°C. Monitor the A₃₄₀nm until constant, using a suitably
thermostatted spectrophotometer. Then add:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (NDPK)</td>
<td>0.05</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>-----</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A₃₄₀nm for approximately 5 minutes. Obtain
the ΔA₃₄₀nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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
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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 µmole 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 β-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:


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 µmole 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 µmole 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.