Enzymatic Assay of PYRUVATE KINASE  
(EC 2.7.1.40)

**PRINCIPLE:**

\[
\text{Phospho(enol)pyruvate} + \text{ADP} \xrightarrow{\text{Pyruvate Kinase}} \text{Pyruvate} + \text{ATP} \quad \text{Mg}^{2+}, \text{K}^+ \\
\text{Pyruvate} + \text{\beta-NADH} \xrightarrow{\text{L-Lactic Dehydrogenase}} \text{L-Lactate} + \text{\beta-NAD}
\]

Abbreviations used:
ADP = Adenosine 5'-Diphosphate  
ATP = Adenosine 5'-Triphosphate  
\text{\beta-NADH} = \text{\beta-Nicotinamide Adenine Dinucleotide, Reduced Form}  
\text{\beta-NAD} = \text{\beta-Nicotinamide Adenine Dinucleotide, Oxidized Form}

**CONDITIONS:**  \( T = 37^\circ C, \ pH = 7.6, A_{340nm}, \ \text{Light path} = 1 \ cm \)

**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. 17 mM Phospho(enol)pyruvate Solution (PEP)  
(Prepare 1 ml in deionized water using Phospho(enol)pyruvate, Trisodium Salt, Hydrate, Sigma Prod. No. P-7002. **PREPARE FRESH.**)

C. 1.3 mM \text{\beta-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (\beta-NADH)}  
(Dissolve the contents of a 5 mg vial of \text{\beta-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129, in the appropriate volume of Reagent A. **PREPARE FRESH.**)

D. 100 mM Magnesium Sulfate Solution (MgSO₄)  
(Prepare 1 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
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REAGENTS: (continued)

E. 44 mM Adenosine Diphosphate Solution (ADP)
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-8146.)

F. l-Lactic Dehydrogenase Solution (LDH)
(Immediately before use, prepare a solution containing 5000 units/ml in cold Reagent A using l-Lactic Dehydrogenase, Sigma Prod. No. L-2500.)

G. Pyruvate Kinase
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of Pyruvate Kinase in cold Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>1.40</td>
<td>1.40</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.80</td>
<td>0.90</td>
</tr>
<tr>
<td>Reagent B (PEP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (ß-NADH)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Reagent D (MgSO₄)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent E (ADP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (LDH)</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (Enzyme Solution) 0.10

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:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\min \text{ Test}} - r_{A_{340\text{nm}}/\min \text{ Blank}})(2.952)(df)}{(6.22)(0.1)}
\]

2.952 = 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

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 2.952 ml reaction mix, the final concentrations are  
39 mM potassium phosphate, 0.58 mM phospho(enol)pyruvate,  
0.11 mM β-nicotinamide adenine dinucleotide, reduced form,  
6.8 mM magnesium sulfate, 1.5 mM adenosine 5'-diphosphate,  
10 units lactic dehydrogenase and 0.015 - 0.030 unit  
pyruvate kinase.

REFERENCE:

Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) Second  
York, NY

NOTES:

1. Lactic Dehydrogenase Unit Definition: One unit will  
reduce 1.0 µmole 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.