Enzymatic Assay of ENOLASE
(EC 4.2.1.11)

PRINCIPLE:

\[
\begin{align*}
\text{Enolase} & : 2\text{-Phosphoglycerate} + \text{H}_2\text{O} \rightarrow \text{PEP} \\
\text{Pyruvate Kinase} & : \text{PEP} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP} \\
\text{L-Lactic Dehydrogenase} & : \text{Pyruvate} + \beta\text{-NADH} \rightarrow \text{L-Lactate} + \beta\text{-NAD}
\end{align*}
\]

Abbreviations used:
- PEP = Phospho(enol)Pyruvate
- ADP = Adenosine 5'-Diphosphate
- ATP = Adenosine 5'-Triphosphate
- $\beta$-NADH = $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form
- $\beta$-NAD = $\beta$-Nicotinamide Adenine Dinucleotide, Oxidized

CONDITIONS: \( T = 25^\circ C, \text{pH} = 7.4, A_{340nm}, \text{Light path} = 1\text{ cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Triethanolamine Buffer, pH 7.4 at 25°C
(Prepare 50 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.4 at 25°C with 1 M NaOH.)

B. 56 mM 2-Phosphoglycerate Solution (DPG)
(Prepare 2 ml in deionized water using d(+)-2-Phosphoglyceric Acid, Sodium Salt, Hydrate, Sigma Prod. No. P-0257.)

C. 7 mM $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form Solution ($\beta$-NADH)
(Dissolve the contents of one 5 mg vial of $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in 1 ml of Reagent A or prepare 1 ml in deionized water using $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium salt, Sigma Prod. No. N-8129. PREPARE FRESH.)

D. 500 mM Magnesium Sulfate with 2 M Potassium Chloride Solution (MgSO$_4$/KCl)
(Prepare 5 ml in deionized water using Magnesium Sulfate, Anhydrous, Sigma Prod. No. M-7506, and Potassium Chloride, Sigma Prod. No. P-4504.)
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REAGENTS: (continued)

E. 20 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-2754. PREPARE FRESH.)

F. PK/LDH Mixed Enzymes
(Use PK/LDH Enzyme Solution, Sigma Prod. No. P-0294)

G. 15 mM Tris HCl with 0.02% (w/v) Bovine Serum Albumin (Enzyme Diluent)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, and Albumin, Bovine, A-4503, or equivalent. Adjust to pH 7.4 at 25°C with 1 M HCl.)

H. Enolase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.5 unit/ml of Enolase in cold Reagent G.)

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>Reagent A (Buffer)</td>
<td>2.39</td>
<td>2.39</td>
</tr>
<tr>
<td>Reagent B (DPG)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (β-NADH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (MgSO₄/KCl)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Reagent E (ADP)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent F (PK/LDH)</td>
<td>0.01</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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G (Enzyme Diluent)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent H (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

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.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Volume (in milliliters) of assay

$df = $ Dilution factor

6.22 = Millimolar extinction coefficient of $\beta$-NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 $\mu$mole of 2-phosphoglycerate to phospho(enol)pyruvate per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 81 mM triethanolamine, 1.9 mM 2-phosphoglycerate, 0.12 mM $\beta$-nicotinamide adenine dinucleotide, reduced form, 25 mM magnesium sulfate, 100 mM potassium chloride, 1.3 mM adenosine 5’-diphosphate, 7 units pyruvate kinase, 10 units $L$-lactic dehydrogenase and 0.025 - 0.05 unit Enolase.

REFERENCES:


NOTES:

1. Contains approximately 700 units/ml of Pyruvate Kinase and 1000 units/ml of Lactic Dehydrogenase.

2. Pyruvate Kinase unit definition: One unit will convert 1.0 $\mu$mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
3. **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)

4. All product 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.