Enzymatic Assay of GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE/
3-PHOSPHOGLYCERIC PHOSPHOKINASE

**PRINCIPLE:**

\[ 3\text{-PGA} + \text{ATP} \xrightarrow{\text{3-PGK}} \text{Glycerate-1,3 Diphosphate} + \text{ADP} \]

\[ \text{Glycerate-1,3 Diphosphate} + \beta\text{-NADH} \xrightarrow{\text{GAPDH}} \text{G-3-P} + \beta\text{-NAD} + \text{P}_i \]

**Abbreviations used:**

- 3-PGA = 3-Phosphoglyceric Acid
- ATP = Adenosine 5'-Triphosphate
- 3-PGK = 3-Phosphoglyceric Phosphokinase
- ADP = Adenosine 5'-Diphosphate
- \( \beta\text{-NADH} = \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form} \)
- GAPDH = Glyceraldehyde-3-Phosphate Dehydrogenase
- G-3-P = Glycerate-3-Phosphate
- \( \beta\text{-NAD} = \beta\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form} \)
- \( \text{P}_i = \text{Inorganic Phosphate} \)

**CONDITIONS:** \( T = 25^\circ \text{C}, \) \( \text{pH} = 7.6, \) \( A_{340\text{nm}}, \) \( \text{Light path} = 1 \text{ cm} \)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Triethanolamine Buffer with 4.0 mM L-Cysteine, and 0.5 mM Ethylenediaminetetraacetic acid, \( \text{pH} \) 7.6 at 25\(^\circ\)C

(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, L-Cysteine, Hydrochloride, Monohydrate, and Ethylenediaminetetraacetic Acid, Di s odi um S al t, Di hydrate. Adjust to \( \text{pH} \) 7.6 at 25\(^\circ\)C with 1 M NaOH.)

B. 100 mM 3-Phosphoglyceric Acid Solution (3-PGA)

(Prepare 2 ml in deionized water using \( d\)(-)-3-Phosphoglyceric Acid, Disodium Salt.)
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REAGENTS: (continued)

C. 100 mM Magnesium Sulfate Solution (MgSO₄)
   (Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate.)

D. 7.0 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
   (Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt
   or dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form
   Disodium Salt, in the appropriate volume of deionized water. PREPARE FRESH.)

E. 34 mM Adenosine 5'-Triphosphate Solution (ATP)
   (Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt.
   PREPARE FRESH.)

F. 3-Phosphoglyceric Phosphokinase Enzyme Solution (3-PGK)
   (Immediately before use, prepare a solution containing
   200 units/ml in cold deionized water using
   3-Phosphoglyceric Phosphokinase.)

G. Glyceraldehyde-3-Phosphate Dehydrogenase Enzyme Solution (GAPDH)
   (Immediately before use, prepare a solution containing
   0.3 - 0.6 unit/ml of Glyceraldehyde-3-Phosphate Dehydrogenase 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>Reagent A (Buffer)</td>
<td>2.45</td>
<td>2.55</td>
</tr>
<tr>
<td>Reagent B (3-PGA)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent C (MgSO₄)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (β-NADH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (ATP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (3-PGK)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ 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>Reaction G (GAPDH)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r_{A_{340\text{nm}}/\text{min}}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total 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 reduce 1.0 µmole of 3-phosphoglycerate to $D$-glyceraldehyde 3-phosphate per minute in a coupled system with 3-phosphoglyceric phosphokinase at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 85 mM triethanolamine, 6.7 mM 3-phosphoglyceric acid, 3.4 mM $L$-cysteine, 0.43 mM ethylenediaminetetraacetic acid, 1.7 mM magnesium sulfate, 0.1 mM $\beta$-nicotinamide adenine dinucleotide, reduced form, 1.1 mM adenosine 5’-triphosphate, 10 units 3-phosphoglyceric phosphokinase and 0.03 - 0.06 unit glyceraldehyde-3-phosphate dehydrogenase.
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NOTES:

1. 3-Phosphoglyceric Phosphokinase unit definition: One unit will convert 1.0 µmole of 1,3-diphosphoglycerate to 3-phosphoglycerate per minute at pH 6.9 at 25°C.

2. All product and stock numbers, unless otherwise indicated, are OUR product and stock numbers.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.