Enzymatic Assay of GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE
(EC 1.2.1.12)

PRINCIPLE:

3-PGA + ATP $\rightarrow$ Glycerate-1,3 Diphosphate + ADP
Glycerate-1,3 Diphosphate + $\beta$-NADH $\rightarrow$ G-3-P + $\beta$-NAD + P$_i$

Abbreviations used:
3-PGA = 3-Phosphoglyceric Acid
ATP = Adenosine 5'-Triphosphate
3-PGK = 3-Phosphoglyceric Phosphokinase
ADP = Adenosine 5'-Diphosphate
$\beta$-NADH = $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form
GAPDH = Glyceraldehyde-3-Phosphate Dehydrogenase
G-3-P = Glyceraldehyde 3-Phosphate
$\beta$-NAD = $\beta$-Nicotinamide Adenine Dinucleotide, Oxidized Form
P$_i$ = Inorganic Phosphate

CONDITIONS:  $T = 25^\circ$C, pH = 7.6, $A_{340nm}$, 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. Prepare fresh.)

B. 100 mM 3-Phosphoglyceric Acid Solution (3-PGA)
(Prepare 2 ml in deionized water using D(-)-3-Phosphoglyceric Acid, Tri(cyclohexylammonium) Salt, Sigma Prod. No. P-8752.)

C. 200 mM L-Cysteine HCl Solution (Cys)
(Prepare 1 ml in deionized water using L-Cysteine Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Neutralize the solution by adding solid Sodium Bicarbonate, Sigma Prod. No. S-8875. PREPARE FRESH.)
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REAGENTS: (continued)

D. 100 mM Magnesium Sulfate Solution (MgSO₄)
(Prepare 10 ml in deionized water using Magnesium
Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

E. 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,
Sigma Prod. No. N-8129 or dissolve the contents of one
5 mg vial of β-Nicotinamide Adenine Dinucleotide,
Reduced Form, Disodium Salt, Sigma Stock No. 340-105,
in the appropriate volume of deionized water. PREPARE
FRESH.)

F. 34 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 1 ml in deionized water using Adenosine
5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-
5394. PREPARE FRESH.)

G. 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, Sigma Prod. No. P-
7634.)

H. 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>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.40</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent B (3-PGA)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent C (Cys)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent D (MgSO₄)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (β-NADH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent F (ATP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent G (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 \( A_{340nm} \) until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction H (Enzyme Solution)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

3 = Volume (in milliliters) of assay
df = Dilution factor
0.1 = Volume (in milliliter) of enzyme used
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm

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

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

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 83 mM triethanolamine, 6.7 mM 3-phosphoglyceric acid, 3 mM L-cysteine, 2 mM magnesium sulfate, 0.1 mM β-NADH, 1.1 mM ATP, 10 units 3-phosphoglyceric phosphokinase and
0.03 - 0.06 unit glyceraldehyde-3-phosphate dehydrogenase.
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NOTES:

1. Not to be used to assay activity of Glyceraldehyde-3-Phosphate Dehydrogenase, from Bacillus Stearothermophilus, Sigma Prod. No. G-5892.

2. 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.

3. 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.