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

**PRINCIPLE:**

3-PGA + ATP $\overset{3-PGK}{\longrightarrow}$ Glycerate-1,3 Diphosphate + ADP

Glycerate-1,3 Diphosphate + β-NADH $\overset{GAPDH}{\longrightarrow}$ G-3-P + β-NAD + P$_i$

Abbreviations used:

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

**CONDITIONS:** T = 30°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 30°C
   (Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 30°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, 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 ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
   (Prepare 1 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of one 5 mg vial of ß-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 100 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 0.2 - 0.4 unit/ml of Glyceraldehyde-3-Phosphate Dehydrogenase in cold Reagent A.)¹

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:²

<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 F (ATP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (MgSO₄)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent E (ß-NADH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent C (Cys)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Then add:

| Reagent G (3-PGK)     | 0.05      | 0.05      |

¹ Revised: 08/31/94
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 30°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 (GAPDH)</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:

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

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 assay

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

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

UNIT DEFINITION:

One unit will reduce 1.0 \( \mu \)mole of 3-phosphoglycerate to \( D \)-glyceraldehyde 3-phosphate per minute in a coupled system with 3-phosphoglyceric phosphokinase at pH 7.6 at 30°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 \( \beta \)-nicotinamide adenine dinucleotide, reduced form, 1.1 mM adenosine 5'-triphosphate, 5 units 3-phosphoglyceric phosphokinase and 0.02 - 0.04 unit glyceraldehyde-3-phosphate dehydrogenase.
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REFERENCE:


NOTES:

1. Adjust the concentration of the enzyme so that the \( \text{A}_{340\text{nm}}/\text{minute} \) is less than 0.09.

2. Do not change the order in which the reagents are added to the cuvettes.

3. 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.

4. This assay is based on the cited reference.

5. 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.