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

PRINCIPLE:
3-PGA + ATP $\rightarrow_{3-PGK}$ Glycerate-1,3 Diphosphate + ADP
Glycerate-1,3 Diphosphate + $\beta$-NADH $\rightarrow_{GAPDH}$ 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: $T = 30^\circ C$, $pH = 7.6$, $A_{340nm}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Triethanolamine Buffer, pH 7.6 at 30°C
   (Prepare 100 ml in deionized water using Triethanolamine Hydrochloride.
   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.)

C. 200 mM L-Cysteine HCl Solution (Cys)
   (Prepare 1 ml in deionized water using L-Cysteine Hydrochloride, Monohydrate. Neutralize the solution
   by adding solid Sodium Bicarbonate. 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.)

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, N-8129 or dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, 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. 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.)

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.)

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 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 |
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 30°C. Monitor the A\textsubscript{340nm} 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 H (GAPDH)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

- 3 = Total volume (in milliliters) of assay
- df = Dilution factor
- 6.22 = Millimolar extinction coefficient of β-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 µmole 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 β-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 \(A_{340}\text{nm/minute}\) 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 \(\mu\)mole 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.

This procedure is for informational purposes.