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

**PRINCIPLE:**

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

Glycerate-1,3 Diphosphate + ß-NADH $\xrightarrow{\text{GAPDH}}$ G-3-P + ß-NAD + $P_i$

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

**CONDITIONS:** T = 30°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, 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 ($MgSO_4$)
(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]

| | Test | Blank |
|---|---|---|
| Reagent A (Buffer) | 2.40 | 2.50 |
| Reagent B (3-PGA) | 0.20 | 0.20 |
| Reagent F (ATP) | 0.10 | 0.10 |
| Reagent D ($MgSO_4$) | 0.05 | 0.05 |
| Reagent E (ß-NADH) | 0.05 | 0.05 |
| Reagent C (Cys) | 0.05 | 0.05 |

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 $A_{340nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | Test | Blank |
|---|---|---|
| Reaction H (GAPDH) | 0.10 | ------ |

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

**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-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 ß-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:**

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd edition, Volume I, 466-467, Academic Press, Inc., New York, NY

**NOTES:**

1. Adjust the concentration of the enzyme so that the $?A_{340nm}$/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.