

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

PRINCIPLE:

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

Glycerate-1,3 Diphosphate + β -NADH $\xrightarrow{\text{GAPDH}}$ 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_{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 (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 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

	<u>Test</u>	<u>Blank</u>
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 (β-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 $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reaction H (GAPDH)	0.10	-----

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ 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 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:

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 $\Delta A_{340\text{nm}}$ /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 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.