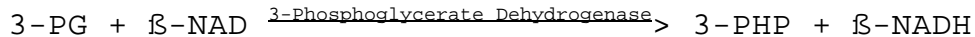


**Enzymatic Assay of 3-PHOSPHOGLYCERATE DEHYDROGENASE
(EC 1.1.1.95)**

PRINCIPLE:



Abbreviations used:

3-PG = D(-)3-Phosphoglycerate

3-PHP = D(-)3-Phosphohydroxypyruvate

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH = 9.0, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1000 mM Tris Buffer, pH 9.0 at 25°C
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 9.0 at 25°C with 1 M HCl.)
- B. 217 mM D(-)3-Phosphoglycerate Solution (3-PG)
(Prepare 1 ml in deionized water using D(-)3-Phosphoglyceric Acid, Disodium Salt, Sigma Prod. No. P-0259.)
- C. 1000 mM Hydrazine Hydrate Solution, pH 9.0 at 25°C
(Hydrazine)
(Prepare 8 ml in deionized water using Hydrazine Hydrate, Sigma Prod. No. H-0883. Adjust to pH 9.0 at 25°C with 1 M HCl.)
- D. 10 mM Ethylenediaminetetraacetic Acid Solution, pH 9.0 at 25°C (EDTA)
(Prepare 5 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 9.0 at 25°C with 100 mM NaOH.)

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REAGENTS: (continued)

- E. 49 mM Reduced Glutathione Solution (GSH)
(Prepare 2 ml in deionized water using Glutathione, Reduced Form, Free Acid, Sigma Prod. No. G-4251.)
- F. 29 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)
- G. 3-Phosphoglycerate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of 3-Phosphoglycerate Dehydrogenase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.00
Reagent B (3-PG)	0.10	0.10
Reagent C (Hydrazine)	1.00	1.00
Reagent D (EDTA)	0.50	0.50
Reagent E (GSH)	0.20	0.20
Reagent F (β -NAD)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized Water	-----	0.10
Reagent G (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase 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.

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CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}}}{(6.22) (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

RM = Reaction Mix

UNIT DEFINITION:

One unit will convert 1.0 μ mole of 3-phosphoglycerate to phosphohydroxypyruvate per minute at pH 9.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 333 mM Tris, 7.2 mM D(-)3 phosphoglycerate, 333 mM hydrazine, 1.7 mM EDTA, 3.3 mM glutathione, 0.97 mM β -NAD and

0.015 - 0.030 unit 3-phosphoglycerate dehydrogenase.

REFERENCE:

Willis, J.E., and Sallach, H.J. (1964) *Biochimica et Biophysica Acta* **81**, 39-54.

NOTES:

1. This assay is a modification of the procedure described in the cited reference.
2. 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.