Enzymatic Assay of 3-PHOSPHOGLYCERATE DEHYDROGENASE (EC 1.1.1.95)

PRINCIPLE:

3-PG + ß-NAD $\xrightarrow{\text{3-Phosphoglycerate Dehydrogenase}}$ 3-PHP + ß-NADH

Abbreviations used:
3-PG = $_D$(-)3-Phosphoglycerate
3-PHP = $_D$(-)3-Phosphohydroxypyruvate
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH = 9.0, A_{340nm}, 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 ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 1 ml in deionized water using ß-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:

	Test	Blank
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 (ß-NAD)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} 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_{340nm} for approximately 5 minutes. Obtain the $r A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

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

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will convert 1.0 µmole 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 ß-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.