Enzymatic Assay of GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE/ 3-PHOSPHOGLYCERIC PHOSPHOKINASE

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 = 25°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Triethanolamine Buffer with 4.0 mM $_L$-Cysteine, and 0.5 mM Ethylendiaminetetraacetic acid, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502, $_L$-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 7.6 at 25°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, Disodium Salt, Sigma Prod. No. P-0259.)

REAGENTS: (continued)

C. 100 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)

D. 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, Stock No. 340-105, in the appropriate volume of deionized water. **PREPARE FRESH**.)

E. 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**.)

F. 3-Phosphoglyceric Phosphokinase Enzyme Solution (3-PGK)
(Immediately before use, prepare a solution containing 200 units/ml in cold deionized water using 3-Phosphoglyceric Phosphokinase, Sigma Prod. No. P-7634.)

G. Glyceraldehyde-3-Phosphate Dehydrogenase Enzyme Solution (GAPDH)
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Glyceraldehyde-3-Phosphate Dehydrogenase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.45	2.55
Reagent B (3-PGA)	0.20	0.20
Reagent C ($MgSO_4$)	0.05	0.05
Reagent D (ß-NADH)	0.05	0.05
Reagent E (ATP)	0.10	0.10
Reagent F (3-PGK)	0.05	0.05

PROCEDURE: (continued)

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

	Test	Blank
Reaction G (GAPDH)	0.10	------

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

CALCULATIONS:

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

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 enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

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 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 85 mM triethanolamine, 6.7 mM 3-phosphoglyceric acid, 3.4 mM L-cysteine, 0.43 mM ethylenediaminetetraacetic acid,
1.7 mM magnesium sulfate, 0.1 mM ß-nicotinamide adenine dinucleotide, reduced form, 1.1 mM adenosine 5'-triphosphate, 10 units 3-phosphoglyceric phosphokinase and 0.03 - 0.06 unit glyceraldehyde-3-phosphate dehydrogenase.

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NOTES:

1. 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.

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.