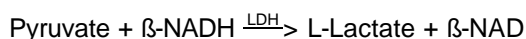
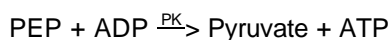
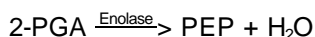


**Enzymatic Assay of PHOSPHOGLYCERATE MUTASE
(EC 5.4.2.1)**

PRINCIPLE:



Abbreviations used:

3-PGA = D(-)3-Phosphoglyceric Acid

DPGA = 2,3-Diphospho-D-Glyceric Acid

2-PGA = 2-Phosphoglyceric Acid

PEP = Phospho(enol)pyruvate

ADP = Adenosine 5'-Diphosphate

PK = Pyruvate Kinase

ATP = Adenosine 5'-Triphosphate

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

LDH = L-Lactic Dehydrogenase

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

CONDITIONS: T = 25°C, pH 7.6, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Triethanolamine Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. 200 mM 3-Phosphoglyceric Acid Solution (3-PGA)
(Prepare 1 ml in deionized water using D(-)3-Phosphoglyceric Acid, Trisodium Salt, Sigma Prod. No. P-0769.)
- C. 21 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 1 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-6521. **PREPARE FRESH.**)

**Enzymatic Assay of PHOSPHOGLYCERATE MUTASE
(EC 5.4.2.1)**

REAGENTS:

- D. 40 mM 2,3-Diphosphoglyceric Acid Solution (DPGA)
(Prepare 1 ml in deionized water using 2,3-Diphospho-D-Glyceric Acid, Pentacyclohexylammonium Salt, Sigma Prod. No. D-9134. **PREPARE FRESH.**)
- E. 6.4 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(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 Reagent A.)
- F. 50 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 2 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- G. 2 M Potassium Chloride Solution (KCl)
(Prepare 2 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)
- H. PK/LDH Enzymes² (PK/LDH)
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)
- I. Enolase Enzyme Solution (Enolase)
(Immediately before use, prepare a solution containing 100 units/ml of Enolase, Sigma Prod No. E-0379, in cold deionized water.)
- J. Phosphoglycerate Mutase Enzyme Solution (Phosgly Mutase)
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Phosphoglycerate Mutase in cold Reagent A.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	22.00
Reagent B (3-PGA) 1.00	
Reagent C (ADP)	1.00
Reagent D (DPGA)	1.00
Reagent E (β -NADH)	0.70
Reagent F ($MgSO_4$) 1.50	
Reagent G (KCl)	1.50

**Enzymatic Assay of PHOSPHOGLYCERATE MUTASE
(EC 5.4.2.1)**

PROCEDURE:

Mix by swirling and adjust to pH 7.6 at 25°C with either 1 M NaOH or 1 M HCl, if necessary.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.87	2.87
Reagent H (PK/LDH)	0.02	0.02
Reagent I (Enolase)	0.03	0.03

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

Reagent J (Phosgly Mutase)	0.10	-----	
Reagent A (Buffer)	-----	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:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3.02)(df)}{(6.22)(0.1)}$$

3.02 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of 3-phosphoglycerate to 2-phosphoglycerate per minute at pH 7.6 at 25°C in the presence of 1.3 mM 2,3-diphosphoglycerate.

**Enzymatic Assay of PHOSPHOGLYCERATE MUTASE
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FINAL ASSAY CONCENTRATIONS:

In a 3.02 ml reaction mix, the final concentrations are 79 mM triethanolamine, 6.6 mM D(-)-3-phosphoglyceric acid, 0.70 mM adenosine 5'-diphosphate, 1.3 mM 2,3-diphospho-D-glyceric acid, 0.15 mM β -nicotinamide adenine dinucleotide, reduced form, 2.5 mM magnesium sulfate, 99 mM potassium chloride, 14 units pyruvate kinase, 20 units L-lactic acid dehydrogenase, 3 units enolase, and 0.03 - 0.06 unit phosphoglycerate mutase.

REFERENCES:

Oesper, P. (1955) *Methods in Enzymology*, Volume 1, 423-425

Sutherland, E.W., Posternak, T., and Cori, C.F. (1949) *Journal of Biological Chemistry* **181**, 153-159

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume I, 503-504, Academic Press, New York, NY

NOTES:

1. 2,3-Diphospho-D-Glyceric Acid is a coenzyme in this reaction.
2. Contains not less than 700 units/ml of Pyruvate Kinase and 1000 units/ml of L-Lactic Dehydrogenase.
3. Pyruvate Kinase Unit Definition: One unit will convert 1.0 μ mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 25°C.
4. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
5. This assay is based on the cited references.
6. 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.