

Enzymatic Assay of PHOSPHOGLUCOSE ISOMERASE¹
(EC 5.3.1.9)

PRINCIPLE:

D-Fructose 6-Phosphate $\xrightarrow{\text{PGI}}$ D-Glucose 6-Phosphate

D-Glucose 6-Phosphate + β -NADP $\xrightarrow{\text{G-6-PDH}}$ 6-Phosphogluconate + β -NADPH

Abbreviations used:

PGI = Phosphoglucose Isomerase

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate,
Oxidized Form

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

CONDITIONS: T = 25°C, pH = 7.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 250 mM Glycylglycine Buffer, pH 7.4 at 25°
(Prepare 100 ml in deionized water using Glycylglycine, Free Base, Sigma Prod. No. G-1002. Adjust the pH to 7.4 with 1 M NaOH.)
- B. 100 mM D-Fructose 6-Phosphate Solution (F-6-P)
(Prepare 1 ml in deionized water using D-Fructose 6-Phosphate, Disodium, Sigma Prod. No. F-3627.)
- C. 20 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (NADP)
(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. **PREPARE FRESH.**)
- D. 100 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

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REAGENTS: (continued)

- E. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)
 (Immediately before use, prepare a solution containing 50 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-6378, in cold deionized water.)
- F. Phosphoglucose Isomerase Enzyme Solution (PGI)
 (Immediately before use, prepare a solution containing 0.3 - 0.7 unit/ml in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	2.00	2.00
Reagent A (Buffer)	0.50	0.50
Reagent B (F-6-P)	0.10	0.10
Reagent C (NADP)	0.10	0.10
Reagent D (MgCl ₂)	0.10	0.10
Reagent E (G-6-PDH)	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:

Reagent F (PGI)	0.10	-----
Deionized Water	-----	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.

CALCULATIONS:

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

- 3 = Total volume (in milliliters) of assay
- df = Dilution factor
- 6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm
- 0.1 = Volume (in milliliter) of enzyme used

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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will convert 1.0 μ mole of D-fructose 6-phosphate to D-glucose 6-phosphate per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 42 mM glycylglycine, 3.3 mM D-fructose 6-phosphate, 0.67 mM β -nicotinamide adenine dinucleotide phosphate, 3.3 mM MgCl₂, 5.0 units glucose-6-phosphate dehydrogenase and 0.03 - 0.07 unit phosphoglucose isomerase.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.) Volume 1, 2nd ed., 501-503, Academic Press, Inc., New York, NY

NOTES:

1. This assay procedure is not to be used to assay the activities of Phosphoglucose Isomerase from *Bacillus stearothermophilus*, Sigma Prod. No. P-5538, and Phosphoglucose Isomerase, Insoluble Enzyme attached to Polyacrylamide, Sigma Prod. No. P-4135.
2. Glucose-6-Phosphate Dehydrogenase unit definition: One unit will oxidize 1.0 μ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25°C.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.