

**Enzymatic Assay of PHOSPHOGLUCOSE ISOMERASE
(EC 5.3.1.9)
from Bacillus stearothermophilus**

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 = 30°C, pH = 9.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 9.0 at 30°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust the pH to 9.0 with 1 M HCl.)
- B. 100 mM D-Fructose 6-Phosphate Solution (F-6-P)
(Prepare 1 ml in deionized water using D-Fructose 6-Phosphate, Disodium Salt, Sigma Prod. No. F-3627.)
- C. 23 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.**)

**Enzymatic Assay of PHOSPHOGLUCOSE ISOMERASE
(EC 5.3.1.9)
from Bacillus stearothermophilus**

REAGENTS: (continued)

- D. 50 mM Tris HCl Buffer, pH 8.5 at 30°C (Enz Dil)
(Prepare 50 ml in deionized water using Trizma Base,
Sigma Prod. No. T-1503. Adjust to pH 8.5 at 30°C with
1 M HCl.)
- E. Glucose-6-Phosphate Dehydrogenase Enzyme
Solution (G-6-PDH)
(Immediately before use, prepare a solution containing
1000 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
0.2 - 0.4 unit/ml in Reagent D.)

PROCEDURE:

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

Reagent A (Buffer)	18.96
Reagent B (F-6-P)	0.60
Reagent C (NADP)	0.40

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.994	2.994
Reagent E (G-6-PDH)	0.006	0.006

Mix by inversion and equilibrate to 30°C. Monitor the
 $A_{340\text{nm}}$ until constant, using a suitably thermostatted
spectrophotometer. Then add:

Reagent F (PGI)	0.10	-----
Reagent D (Enz Dil)	-----	0.10

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

**Enzymatic Assay of PHOSPHOGLUCOSE ISOMERASE
(EC 5.3.1.9)
from Bacillus stearothermophilus**

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{340\text{nm}}/\text{min Test} - \text{r } A_{340\text{nm}}/\text{min Blank})(3.1)(\text{df})}{(6.22)(0.1)}$$

3.1 = 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

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

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

UNIT DEFINITION:

One unit will convert 1.0 μ mole of D-fructose 6-phosphate to D-glucose 6-phosphate per minute at pH 9.0 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 93 mM Tris, 2.9 mM D-fructose 6-phosphate, 0.44 mM β -nicotinamide adenine dinucleotide phosphate, 6.0 units glucose-6-phosphate dehydrogenase and 0.02 - 0.04 unit phosphoglucose isomerase.

NOTES:

1. 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.
2. 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.