

## Enzymatic Assay of PHOSPHOMANNOSE ISOMERASE

### PRINCIPLE:

D-Mannose 6-Phosphate  $\xrightarrow{\text{PMI}}$  D-Fructose 6-Phosphate

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

D-Glucose 6-Phosphate +  $\beta$ -NADP  $\xrightarrow{\text{G6PDH}}$  Gluconate-6-P + NADPH

Abbreviations used:

PMI = Phosphomannose Isomerase

PGI = Phosphoglucose Isomerase

G6PDH = Glucose-6-Phosphate Dehydrogenase

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

**CONDITIONS:** T = 25°C, pH 7.6, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 100 mM Triethanolamine HCl 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. 66 mM D-Mannose 6-Phosphate Solution (Mann-6-P)  
(Prepare 10 ml in deionized water using D-Mannose  
6-Phosphate Disodium Salt, Sigma Prod. No. M-6876.)
- C. 13.5 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form, Solution ( $\beta$ -NADP)  
(Prepare 5 ml in deionized water using  $\beta$ -Nicotinamide  
Adenine Dinucleotide Phosphate, Sodium salt, Sigma  
Prod. No. N-0505. **PREPARE FRESH.**)
- D. Phosphoglucose Isomerase Enzyme Solution (PGI)  
(Immediately before use, prepare a solution containing  
1000 units/ml of Phosphoglucose Isomerase, Sigma Prod.  
No. P-2338, in cold deionized water.)

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**REAGENTS:** (continued)

- E. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G6PDH)  
(Immediately before use, prepare a solution containing 100 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-4134, in cold deionized water.)
- F. Phosphomannose Isomerase Enzyme Solution (PMI)  
(Immediately before use, prepare a solution containing 0.075 - 0.150 units/ml of Phosphomannose Isomerase in cold Reagent A.)

**PROCEDURE:**

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

	Test	Blank
Reagent A (Buffer)	2.52	2.52
Reagent B (Mann-6-P)	0.25	0.25
Reagent C (β-NADP)	0.10	0.10
Reagent D (PGI)	0.02	0.02
Reagent E (G6PDH)	0.01	0.01

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

Reagent F (PMI)	0.10	-----
Reagent A (Buffer)	-----	0.10

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

**CALCULATIONS:**

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

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  $\mu$ mole of D-mannose 6-phosphate to D-fructose 6-phosphate per minute at pH 7.6 at 25°C, using a coupled enzyme system with phosphoglucose isomerase and glucose-6-phosphate dehydrogenase.

### FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 87 mM triethanolamine, 5.5 mM D-mannose 6-phosphate, 0.45 mM nicotinamide adenine dinucleotide phosphate, 20 units phosphoglucose isomerase, 1 unit glucose-6-phosphate dehydrogenase and 0.0075 - 0.015 unit phosphomannose isomerase.

### REFERENCES:

Gracy, R. W. and Noltmann, E.A. (1968) *J. Biol. Chem.* **243**, 3161

### NOTES:

1. Glucose-6-Phosphate Dehydrogenase unit definition: One unit will oxidize 1.0  $\mu$ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of  $\beta$ -NADP at pH 7.4 at 25°C.
2. Phosphoglucose Isomerase unit definition: One unit will convert 1.0  $\mu$ mole of fructose 6-phosphate to glucose 6-phosphate per minute at pH 7.4 at 25°C.
3. 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.**