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

PRINCIPLE:

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

\[ \text{D-Glucose 6-Phosphate} + \beta-\text{NADP} \xrightarrow{G-6-PDH} 6-\text{Phosphogluconate} + \beta-\text{NADPH} \]

Abbreviations used:
PGI = Phosphoglucose Isomerase
\(\beta\)-NADPH = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
\(\beta\)-NADP = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
G-6-PDH = Glucose-6-Phosphate Dehydrogenase

CONDITIONS: \(T = 30^\circ\text{C}, \text{pH} = 9.0, A_{340nm}, \text{Light path} = 1 \text{ cm}\)

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 \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate Solution (NADP)
(Prepare 1 ml in deionized water using \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. PREPARE FRESH.)
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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:

<table>
<thead>
<tr>
<th>Reagent A (Buffer)</th>
<th>18.96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (F-6-P)</td>
<td>0.60</td>
</tr>
<tr>
<td>Reagent C (NADP)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.994</td>
<td>2.994</td>
</tr>
<tr>
<td>Reagent E (G-6-PDH)</td>
<td>0.006</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the
A_{340nm} 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_{340nm} for approximately 5 minutes. Obtain the r A_{340nm}/minute
using the maximum linear rate for both the Test and Blank.
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**CALCULATIONS:**

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

- **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 µmole 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 µmole 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.