SIGMA QUALITY CONTROL TEST

PROCEDURE

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:
PGL = 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: \( T = 25^\circ\text{C}, \quad \text{pH} = 7.4, \quad A_{340nm}, \quad \text{Light path} = 1 \text{ cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Glycylglycine Buffer, pH 7.4 at 25°C
   (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.)
Enzymatic Assay of PHOSPHOGLUCOSE ISOMERASE\(^1\)  
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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:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent B (F-6-P)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (NADP)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (MgCl(_2))</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (G-6-PDH)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (PGI)</td>
<td>------</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay  
\(\text{df}\) = Dilution factor  
6.22 = Millimolar extinction coefficient of \(\beta\)-NADPH at 340 nm  
0.1 = Volume (in milliliter) of enzyme used
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(EC 5.3.1.9)

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 µmole 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:


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.

2. 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.

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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