SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of HEXOKINASE¹
(EC 2.7.1.1)

PRINCIPLE:

\[
\text{D-Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{D-Glucose 6-Phosphate + ADP}
\]

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

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
G-6-PDH = Glucose-6-Phosphate Dehydrogenase
\(\beta\text{-NADP} = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form}\)
\(\beta\text{-NADPH} = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form}\)
6-PG = 6-Phospho-D-Gluconate

CONDITIONS: \(T = 25^\circ C, \text{pH} = 7.6, A_{340nm}, \text{Light path} = 1 \text{ cm}\)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Triethanolamine 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. 555 mM D-Glucose Solution
(Prepare 10 ml in Reagent A using D-(+)-Glucose, Anhydrous, Sigma Prod. No. G-8270.)

C. 19 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-2383. \text{PREPARE FRESH.})

D. 100 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
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REAGENTS: (continued)

E. 14 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (β-NADP)
(Dissolve the contents of two 10 mg vials of β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310, in the appropriate volume of deionized water or prepare 10 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. PREPARE FRESH.)

F. Glucose-6-Phosphate Dehydrogenase Enzyme Solution (G-6-PDH)²
(Immediately before use, prepare a solution containing 125 units/ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-4134, in cold Reagent A.)³

G. Hexokinase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Hexokinase in cold deionized water.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent B (D-Glucose)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent C (ATP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (MgCl₂)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent E (β-NADP)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent F (G-6-PDH)</td>
<td>0.02</td>
<td>0.02</td>
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</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A₃₄₀nm until constant, using a suitably thermostatted spectrophotometer. Then add:

<p>| | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent G (Enzyme Solution)</td>
<td>0.05</td>
<td>------</td>
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</tbody>
</table>

Immediately mix by inversion and record the increase in A₃₄₀nm for approximately 5 minutes. Obtain the ΔA₃₄₀nm/minute using the maximum linear rate for both the Test and Blank.
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CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340nm/min} \text{ Test} - \Delta A_{340nm/min} \text{ Blank})(2.57)(df)}{(6.22)(0.05)}
\]

2.57 = Total volume (in milliliters) of assay
\(df\) = Dilution factor
6.22 = Millimolar extinction coefficient of \(\beta\)-NADPH at 340 nm
0.05 = 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 phosphorylate 1.0 \(\mu\)mole of D-glucose per minute at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 2.57 ml reaction mix, the final concentrations are 39 mM triethanolamine, 216 mM D-glucose, 0.74 mM adenosine 5’-triphosphate, 7.8 mM magnesium chloride, 1.1 mM \(\beta\)-nicotinamide adenine dinucleotide phosphate, 2.5 units glucose-6-phosphate dehydrogenase, and 0.025 - 0.05 unit of hexokinase.

REFERENCES:


NOTES:

1. This procedure is not to be used to assay the activity of Hexokinase, Sigma Prod. No. H-3779, Hexokinase, Insoluble enzyme attached to beaded agarose, Sigma Prod. No. H-2005, and Hexokinase, Insoluble enzyme attached to polyacrylamide, Sigma Prod. No. H-8254.
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NOTES

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 β-NADP at pH 7.4 at 25°C.

3. Other types of glucose-6-phosphate dehydrogenase may contain varying amounts of hexokinase as an impurity.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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