Enzymatic Assay of MALTASE
(EC 3.2.1.20)

PRINCIPLE:

\[
\text{Maltose} + \text{H}_2\text{O} \xrightarrow{\text{Maltase}} 2 \text{Glucose}
\]

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

\[
\text{Glucose 6-Phosphate} + \beta-\text{NADP} \xrightarrow{\text{G-6-P-DH}} \text{Gluconate} + \beta-\text{NADPH}
\]

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
\(\beta\)-NADP = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
G-6-P-DH = Glucose 6-Phosphate Dehydrogenase
\(\beta\)-NADPH = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: \(T = 25^\circ\text{C}, \text{pH} 6.0, A_{340\text{nm}}, \text{Light path} = 1\ \text{cm}\)

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 6.0 at 25°C (Phosphate Buffer)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous. Adjust to pH to 6.0 at 25°C with 1 M KOH.)

B. 300 mM Maltose Solution (Maltose)
(Prepare 10 ml in deionized water using Maltose, Monohydrate.)

C. 300 mM Triethanolamine HCl Buffer, pH 7.6 at 25°C (TEA Buffer)
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride. Adjust to pH 7.6 at 25°C with 1 M NaOH.)

D. 100 mM Magnesium Chloride Solution (MgCl\(_2\))
(Prepare 10 ml in deionized water using Magnesium Chloride, 4.9 M Solution.)
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REAGENTS: (continued)

E. 16 mM Adenosine 5'-Triphosphate Solution (ATP)  
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt.)

F. 12 mM β-Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form, Solution (β-NADP)  
(Prepare 10 ml in deionized water using β-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt.)

G. Hexokinase and Glucose-6-Phosphate Dehydrogenase  
Enzyme Solution (Hex/G-6-PDH)  
(Immediately before use, prepare a solution containing 200 Hexokinase units/ml of Hexokinase and Glucose-6-Phosphate Dehydrogenase in cold Reagent C.)

H. Maltase Enzyme Solution (Maltase)  
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Maltase in cold Reagent A.)

PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Phosphate Buffer)</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Reagent B (Maltose)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 25°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (Maltase)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Phosphate Buffer)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by swirling and incubate at 25°C for exactly 30 minutes. Then place in a boiling water bath for 3 minutes. Cool to room temperature. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>9.00</td>
<td>9.00</td>
</tr>
</tbody>
</table>

Mix by swirling.
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**PROCEDURE:** (continued)

Step 2:

Prepare a reaction cocktail by combining the following reagents (in milliliters):

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (TEA Buffer)</td>
<td>25.00</td>
</tr>
<tr>
<td>D (MgCl₂)</td>
<td>1.00</td>
</tr>
<tr>
<td>E (ATP)</td>
<td>1.00</td>
</tr>
<tr>
<td>F (β-NADP)</td>
<td>1.00</td>
</tr>
<tr>
<td>G (Hex/G-6-PDH)</td>
<td>0.20</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>2.50</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Equilibrate to 25°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted Test from Step 1</td>
<td>0.50</td>
<td>------</td>
</tr>
<tr>
<td>Diluted Blank from Step 1</td>
<td>------</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 5 - 10 minutes until constant, using a suitably thermostatted spectrophotometer. Obtain the final $\Delta A_{340\text{nm}}$ for both the Test and Blank solutions.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}} \text{ Test} - \Delta A_{340\text{nm}} \text{ Blank}) (10)(3)(\text{df})}{(2)(6.22)(0.1)(0.5)(30)}$$

10 = Total volume (in milliliters) of assay in Step 1  
3 = Total volume (in milliliters) of assay in Step 2  
$\text{df} = \text{Dilution factor}$  
2 = Moles of glucose released per mole of maltose  
6.22 = Millimolar extinction coefficient of β-NADPH at 340nm  
0.1 = Volume (in milliliters) of enzyme used in Step 1  
0.5 = Volume (in milliliters) of diluted test used in Step 2  
30 = Reaction time (in minutes) of Step 1

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$
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CACLUTIONS: (continued)

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of maltose to 2.0 µmoles of D-glucose per minute at pH 6.0 at 25°C (liberated glucose determined at pH 7.6).

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 25 mM potassium phosphate, 150 mM maltose and 0.025 - 0.050 unit maltase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

2. Hexokinase Unit Definition: One unit will phosphorylate 1.0 µmole of D-glucose per minute at pH 7.6 at 25°C.

3. Glucose-6-Phosphate Dehydrogenase: 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.

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

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.