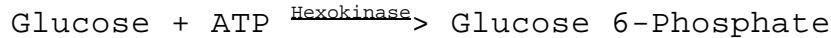
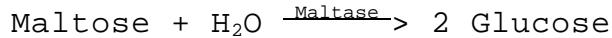


## Enzymatic Assay of MALTASE (EC 3.2.1.20)

### PRINCIPLE:



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°C, pH 6.0,  $A_{340\text{nm}}$ , Light path = 1 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, Sigma Prod. No. P-5379. 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, Sigma Prod. No. M-5885.)
- C. 300 mM Triethanolamine HCl Buffer, pH 7.6 at 25°C (TEA Buffer)  
(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.)
- D. 100 mM Magnesium Chloride Solution ( $\text{MgCl}_2$ )  
(Prepare 10 ml in deionized water using Magnesium Chloride, 4.9 M Solution, Sigma Stock No. 104-20.)

**Enzymatic Assay of MALTASE  
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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, Sigma Prod. No. A-5394.)
- F. 12 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution ( $\beta$ -NADP)  
(Prepare 10 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)
- 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, Sigma Prod. No. H-8629 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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Phosphate Buffer)	0.40	0.40
Reagent B (Maltose)	0.50	0.50

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

Reagent H (Maltase)	0.10	-----
Reagent A (Phosphate Buffer)	-----	0.10

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:

Deionized Water	9.00	9.00
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Mix by swirling.

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**PROCEDURE:** (continued)

Step 2:

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

Reagent C (TEA Buffer)	25.00
Reagent D (MgCl <sub>2</sub> )	1.00
Reagent E (ATP)	1.00
Reagent F (β-NADP)	1.00
Reagent G (Hex/G-6-PDH)	0.20

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.50	2.50

Equilibrate to 25°C. Then add:

Diluted Test from Step 1	0.50	-----
Diluted Blank from Step 1	-----	0.50

Mix by inversion and record the increase in A<sub>340nm</sub> for approximately 5 - 10 minutes until constant, using a suitably thermostatted spectrophotometer. Obtain the final ΔA<sub>340nm</sub> 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

df = 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

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

**Enzymatic Assay of MALTASE  
(EC 3.2.1.10)**

**CACULATIONS:** (continued)

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

**UNIT DEFINITION:**

One unit will convert 1.0  $\mu$ mole of maltose to 2.0  $\mu$ moles 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:**

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, Vol. 1, 2nd ed., 459-460.

**NOTES:**

1. This assay is based on the cited reference.
2. Hexokinase Unit Definition: One unit will phosphorylate 1.0  $\mu$ mole of D-glucose per minute at pH 7.6 at 25°C.
3. Glucose-6-Phosphate Dehydrogenase: One unit will oxidize 1.0  $\mu$ mole 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 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.**