



Maltose Quantification Assay Kit

Product No. M4147

Step-by-Step Protocol

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IMPORTANT:

Avoid repeated freeze-thaw cycles of reagents. This kit can detect maltose in various samples depending on your research goals. Samples may need to be diluted in buffer or distilled water if outside the range of the standard curve.

- Use the colorimetric assay when maltose concentrations are in the range of 50 – 500 μM .
- Use the fluorometric assay for detecting lower maltose concentrations in the range of 0 – 50 μM .
- If unsure, start with a colorimetric assay, perform appropriate gradient dilutions, and plot the spectrophotometric data to check for a linear correlation.

Colorimetric Assay Procedure**Preparation:**

- Equilibrate all components to room temperature. Keep on ice or in a refrigerator during experiment.
- Prepare standard curve using the following dilutions:

No.	Maltose Standard	Assay Buffer	Final Concentration (μM)
1	10 μL	190 μL	500
2	8 μL	192 μL	400
3	6 μL	194 μL	300
4	4 μL	196 μL	200
5	2 μL	198 μL	100
6	0 μL	200 μL	0

Procedure:

1. Transfer 10 μL of standards and 10 μL of samples into separate wells of a 96-well microplate.
2. Prepare the reaction mix (93 μL per well) as follows:

Enzyme I	1 μL
EnzyBoost MIX	1 μL
EnzyBright	1 μL
Assay Buffer	90 μL

3. Add 90 μL of the reaction mix to each well containing standards and samples and mix well.
4. Incubate the reaction at 37 °C for 30 minutes, protect from light.
5. Measure the optical density (OD) at 550 – 580 nm.

Fluorometric Assay Procedure

Preparation:

- Equilibrate all components to room temperature. Keep on ice or in a refrigerator during experiment.
- Mix 10 μL of Maltose Standard with 90 μL Assay Buffer to prepare a 1 mM maltose standard.
- Prepare the standard curve using the following dilutions:

No.	1 mM maltose standard	Assay Buffer	Final Concentration (μM)
1	10 μL	190 μL	50
2	8 μL	192 μL	40
3	6 μL	194 μL	30
4	4 μL	196 μL	20
5	2 μL	198 μL	10
6	0 μL	200 μL	0

Procedures:

1. Transfer 10 μL of standards and 10 μL of samples into separate wells of a 96-well microplate.
2. Prepare the reaction mix (93 μL per well) as follows:

Enzyme I	1 μL
EnzyBoost MIX	1 μL
EnzyBright	1 μL
Assay Buffer	90 μL

3. Add 90 μL of the reaction mix to each well containing standards and samples and mix well.
4. Incubate the reaction at 37 °C for 30 minutes, protect from the light.
5. Measure the fluorescence (F) at Ex 520 – 580 nm / Em 585 – 595 nm.

Calculation of Maltose Concentration in Samples

1. Depending on which assay was performed, calculate either the average OD or F of each standard and sample.
2. Subtract the OD or F of the blank (standard #6) from itself and all other standards to get baseline - corrected values.
3. Plot these corrected values with respect to concentration to obtain a linear standard curve. Calculate its slope.
4. Calculate the maltose concentration using the following equation:

$$\text{Maltose} = \frac{\Delta\text{OD}_{\text{sample}} \text{ or } \Delta\text{F}_{\text{sample}}}{\text{Slope}} \times n \text{ (}\mu\text{M)}$$

- $\Delta\text{OD}_{\text{sample}}$ or $\Delta\text{F}_{\text{sample}}$ = Absorbance or fluorescence of the sample subtract from the blank.
- Slope = Slope of the maltose standard curve.
- n = Dilution factor.
- It is necessary to set a control group which does not contain Enzyme I for the sample containing glucose.