Maltose Assay Kit (Colorimetric/Fluorometric)

LS-K132-100 (100 Tests) • **Store at -20°C**

Introduction

Maltose ($C_{12}H_{22}O_{11}$; FW: 342.3), one of the main fuel sources to generate the universal energy molecule ATP, is the major disaccharide that is generated from hydrolysis of starch in food. Maltose contains two glucose units joined by a α-1,4-glycosidic linkage, which can be easily converted to two glucoses by α-D-glucosidase. The generated glucose can be specifically oxidized to produce a product that interacts with the probe to generate color and fluorescence. Thus, maltose can be determined by either colorimetric (spectrophotometry at λ = 570 nm) or fluorometric (Ex/Em = 535/587 nm) methods. The kit provides a fast, easy and sensitive method for quantifying maltose in various biological samples (e.g. serum, plasma, body fluids, food, growth medium, etc.).

Components

Component	K132-100 100 Tests	Cap Code
Maltose Assay Buffer	25 ml	WM
Maltose Probe (in DMSO, Anhydrous)	0.2 ml	Red
α-D-Glucosidase (Lyophilized)	1 vial	Blue
Enzyme Mix (Lyophilized)	1 vial	Green
Maltose Standard (100 nmol/µl)	100 µl	Yellow

Storage Conditions and Reagents Preparation

Store kit at -20°C, protect from light. Briefly centrifuge vials prior to opening. Allow reagents to warm to room temperature before use, but keep enzymes cold.

- Maltose Probe: Briefly warm at 37°C for 1-2 min to dissolve. Mix well. Store at -20°C, protected from light and moisture. Use within two months.
- α-D-Glucosidase & Enzyme Mix: Dissolve in 220 µl Assay Buffer individually by pipetting up and down. Aliquot and store at -20°C. Use within two months.

Assay Procedure

1. Standard Curve Preparations:

   For the colorimetric assay, dilute the 100 nmol/µl Maltose Standard to 0.5 nmol/µl by adding 5 µl of the Maltose Standard to 995 µl of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 1, 2, 3, 4, 5 nmol/well of Maltose Standard.

   Fluorometric assay is ~10 times more sensitive than the colorimetric assay. For fluorometric assay, dilute the Maltose Standard solution to 0.05 nmol/µl by adding 5 µl of the Maltose Standard to 995 µl of Assay Buffer, mix well. Then take 20 µl into 180 µl of Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Bring volume to 50 µl/well with Assay Buffer to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of the Maltose Standard.

2. Sample Preparations: Prepare test samples in 50 µl/well with Assay Buffer in a 96-well plate. Serum can be directly diluted in the Assay Buffer. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

3. Conversion of maltose to glucose: Add 2 µl of Glucosidase* into each standard and sample well, mix well.

   *Note: Glucose can generate background in the maltose assay. However, the glucose background can be easily eliminated by doing a glucose background control in the absence of Glucosidase. If glucose is present in your samples, prepare two wells for each sample. Add 2 µl of α-D-Glucosidase into one well, and add 2 µl of assay buffer into the other well as glucose background control.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

   - 46 µl Assay Buffer
   - 2 µl Probe*
   - 2 µl Enzyme Mix

   *Note: In the fluorometric Assay, using 0.4 µl probe for each reaction will significantly decrease fluorescence background, and thus increase fluorescence signal/noise ratio.

5. Mix well. Add 50 µl of the Reaction Mix to each well containing the Maltose Standard or test samples. Mix well.

6. Incubate the reaction for 60 min at 37°C, protect from light.

7. Measure OD 570 nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.

8. Calculations: Correct background by subtracting the value derived from the 0 maltose standard from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Subtract glucose background from maltose samples. Plot the standard curve. Apply sample readings to the standard curve. The concentration can then be calculated:

   C = Sa/Sv nmol/ml, or µmol/ml, or mM

   Where: Sa is the sample amount from maltose standard curve, Sv is the sample volume added in sample wells.

   Maltose molecular weight: 342.3; Glucose: 180.2.

Sample Data

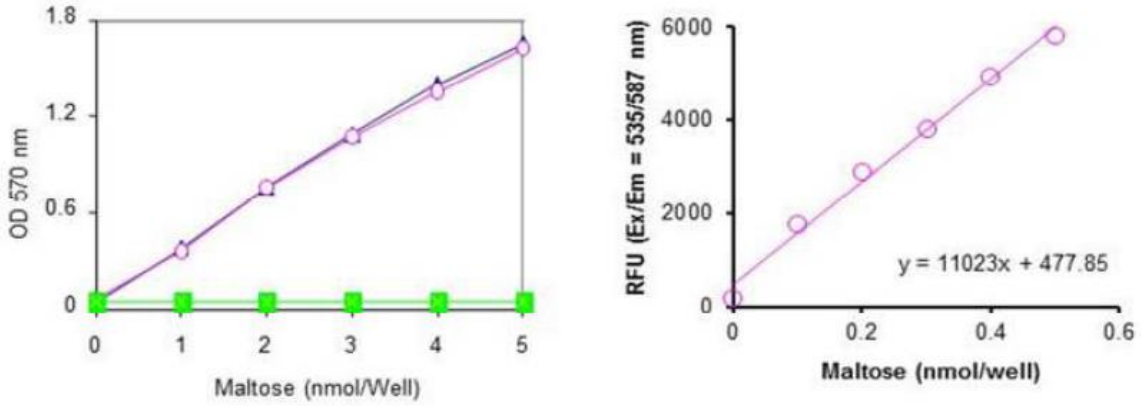

Figure Legend: Maltose Standard Curve. Assays were performed following kit instructions. The open circle is maltose with α-D-Glucosidase, the solid square is maltose without α-D-Glucosidase, and the triangle is free glucose (divided by 2).

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