Technical Bulletin

Maltose Assay Kit

Catalogue number MAK513

Product Description

Maltose ($C_{12}H_{22}O_{11}$) is a disaccharide, composed of two glucose units linked by an α bond. It is produced from the hydrolysis of glycogen or starch, serving as a source of energy for plants and animals. Maltose can be found in foods such as grains, and other processed products.

The Maltose Assay Kit provides a simple, one step assay for measuring maltose. In this assay, maltose is converted to two glucoses, which are then oxidized to form a colored product. The color intensity of the product at 570 nm or fluorescence intensity at λ_{Ex}=530 nm/ λ_{Em}=585 nm is directly proportional to the maltose concentration in the sample.

The linear detection range of the kit 2 to 500 µM maltose for the colorimetric assay and 1 to 50 µM for the fluorometric assay. The kit is suitable for maltose activity determination in various biological samples such as serum, urine, food, and beverages

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalog Number MAK513A
- Assay Buffer 10 mL
  Catalog Number MAK513A
- Enzyme A 120µL
  Catalog Number MAK513C
- Enzyme Mix 120 µL
  Catalog Number MAK513D
- Standard (5 mM Maltose) 1 mL
  Catalog Number MAK513E

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Sample Preparation

All Samples and Standards should be run in duplicate. Clear and slightly colored Samples can be assayed directly.

Serum can be assayed directly. Centrifuge first to remove any particulates. Appropriate dilution in purified water may be required.

Urine Samples can be assayed directly. Centrifuge first to remove any particulates. Urine Samples require the use of an internal standard (see below).

All Samples

1. Transfer 10 µL of each Sample into separate wells of a 96-well plate. For unknown samples, perform a pilot experiment by testing several dilutions to

ensure the readings are within the linear detection range of the kit.

2. To each Sample well, add 5 µL of purified water.

Sample Blank

1. To each Sample Blank well, add 5 µL of purified water.

Internal Standard (required for urine samples)

1. For colorimetric assay, to each Internal Standard well, add 5 µL of 500 µM Maltose Standard (see Colorimetric Standard Curve Preparation section for preparation instructions).
2. For fluorometric assay, to each Internal Standard well, add 5 µL of 50 µM Maltose Standard (see Fluorometric Standard Curve Preparation section for preparation instructions).

Colorimetric Standard Curve Preparation

1. Prepare a 500 µM Maltose Standard by mixing 50 µL of the 5 mM Standard with 450 µL of purified water.
2. Prepare standards in 1.5 mL centrifuge tubes with purified water according to Table 1.

Table 1.
Preparation of Maltose Colorimetric Standards

Well No.	500 µM Standard	Purified Water	Maltose (µM)
1	100 µL	0 µL	500
2	60 µL	40 µL	300
3	30 µL	70 µL	150
4	0 µL	100 µL	0

3. Mix well and transfer 10 mL of each Standard into separate wells of a clear 96 well plate.

Fluorometric Standard Curve Preparation

1. Prepare standards according to Colorimetric Standard Curve Preparation section.
2. Mix 10 µL of the standards from Colorimetric Procedure with 90 µL of purified according to Table 2.

Table 2.
Preparation of Fluorometric Maltose Standards

Well	Colorimetric Standard	Purified Water	Maltose (µM)
1	10 µL of 500 µM Std	90 µL	50
2	10 µL of 300 µM Std	90 µL	30
3	10 µL of 150 µM Std	90 µL	15
4	-	100 µL	0

3. Mix well and transfer 10 mL of each Standard into separate wells of a black 96 well plate

Working Reagent Preparation

Mix enough reagent for the number of assays to be performed. For each Sample, Internal Standard, and Standard well, prepare 98 mL of Working Reagent according to Table 3. Prepare 97 mL of Blank Working Reagent for each Sample Blank well according to Table 3.

Table 3.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	95 µL	95 µL
Enzyme A	1 µL	-
Enzyme Mix	1 µL	1 µL
Dye Reagent	1 µL	1 µL

Transfer 90 µL of Working Reagent into each Sample, Internal Standard, and Standard well. Transfer 90 µL of Blank Working Reagent into each Sample Blank well. Tap plate to mix.

Measurement

1. Incubate the plate for 60 minutes at room temperature.
2. Measure the optical density at 570 nm or fluorescence intensity at λ_{Ex} = 530 nm/ λ_{Em}=585nm.

   Note: The volume of the internal standard is 2× lower than the sample volume; thus, the internal standard concentration should be divided by 2. If the calculated maltose concentration is >500 µM for the colorimetric assay, or >50 µM for the fluorometric assay, dilute sample in purified water and repeat assay. Multiply result by the dilution factor (*DF*).

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔF or ΔOD against the standard concentrations.
3. Determine the slope and calculate the maltose concentration of samples using the below equation:

   Maltose (µM) =

$$\frac{\mathrm{R_{Sample}} - \mathrm{R_{Blank}}}{\mathrm{Slope}(\mu\mathrm{M}^{-1})} \times DF$$

4. If an Internal Standard was used, the sample maltose concentration is calculated as follows:

   Maltose (µM) =

$$\frac{R_{Sample} - R_{Blank}}{R_{Standard} - R_{Sample}} \; x \; \frac{[Standard\}}{2} \; x \; DF$$

where:

R_{Sample} = Fluorescence intensity (F) or OD reading of Sample

R_{Blank} = Fluorescence intensity (F) or OD reading of Sample Blank

$R_{Standard}$ = Fluorescence intensity (F) or OD reading of Internal Standard

DF = Sample dilution factor (DF = 1 for undiluted Samples)

[Standard] = Concentration of the Internal Standard (500 µM for the colorimetric assay and 50 µM for the fluorometric assay)

2 = The volume of the internal standard is 2× lower than the sample volume; thus, the internal standard concentration should be divided by 2

Note: If the calculated maltose concentration is >500 µM for the colorimetric assay, or >50 µM for the fluorometric assay, dilute the sample in purified water and repeat the assay. Multiply the result by the dilution factor (*DF*).

Conversions: 1 mM maltose equals 34.23 mg/dL, or 342.3 ppm.

Figure 1.

Typical Colorimetric Maltose Standard Curve

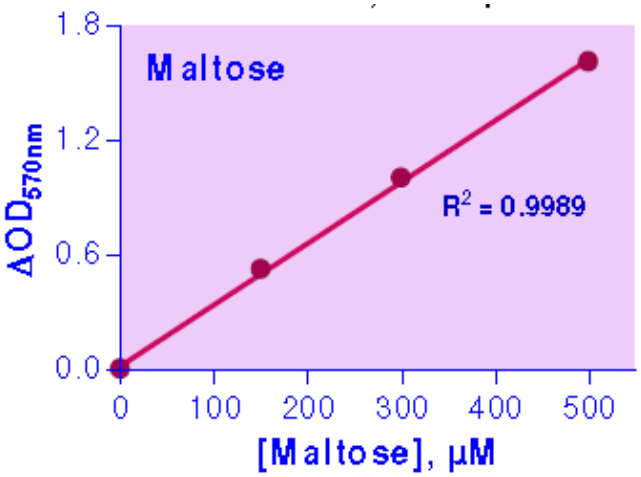
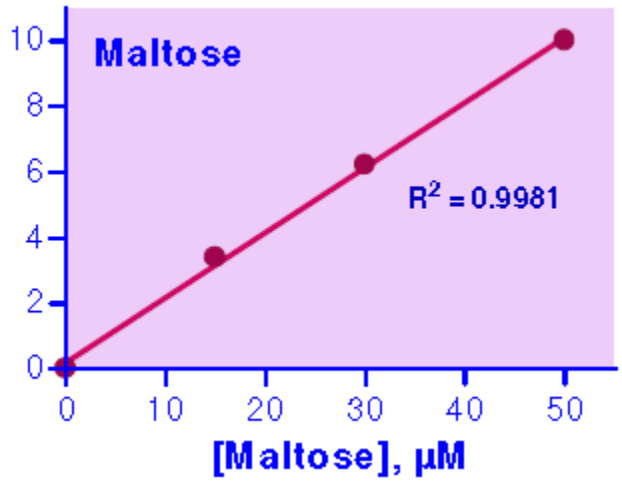

Maltose
R^2 = 0.9989
ΔOD_{570nm}
1.8
1.2
0.6
0.0
0
100
200
300
400
500
[Maltose], µM

Figure 2.


Maltose
R^2 = 0.9981
10
8
6
4
2
0
0
10
20
30
40
50
[Maltose], µM

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