Maltose Assay Kit (Colorimetric)

Maltose Assay Kit (Colorimetric) is a detection kit for the quantification of Maltose in serum, urine, food and beverages.

Catalog number: ARG82180

Package: 100 tests

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INTRODUCTION

Maltose (/ˈmɔːltoʊs/ or /ˈmɔːltoʊz/), also known as maltobiose or malt sugar, is a disaccharide formed from two units of glucose joined with an α(1→4) bond. In the isomer isomaltose, the two glucose molecules are joined with an α(1→6) bond. Maltose is the two-unit member of the amylose homologous series, the key structural motif of starch. When alpha-amylase breaks down starch, it removes two glucose units at a time, producing maltose. An example of this reaction is found in germinating seeds, which is why it was named after malt. Unlike sucrose, it is a reducing sugar. [Provide by Wikipedia: Maltose]

PRINCIPLE OF THE ASSAY

This Maltose Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of maltose present in various biological samples such as serum, urine, food and beverages. 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 O.D. 570 nm or fluorescence at λex/em = 530/585 nm is directly proportional to maltose concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on dry ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Enzyme A	120 µL	-20°C
Enzyme Mix	120 µL	-20°C
Dye Reagent	120 µL	-20°C
Standard (5 mM Maltose)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C, and collect the supernatant for assay. Appropriate dilution in distilled water may be required.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C, and collect the supernatant for assay. Appropriate dilution in distilled water may be required.

Urine: centrifuge at 1000 x g for 10 minutes at 4°C, and collect the supernatant for assay. An internal standard is required.

Other samples: Clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor n.

REAGENT PREPARATION

- **Working Reagent:** for each assay, mix 95 µL of Assay Buffer, 1 µL of Enzyme A, 1 µL of Enzyme Mix and 1 µL of Dye Reagent. Prepare immediately before assay.
- **Blank Working Reagent:** for each assay, mix 95 µL of Assay Buffer, 1 µL of Enzyme Mix and 1 µL of Dye Reagent. Prepare immediately before assay.
- **Standards:** mix 50 µL of 5 mM Standard with 450 µL of distilled water (final 500 µM). Dilute standards as follows.

Standard tube	Maltose (µM)	Distilled water (µL)	Standard Premix, 500 µM (µL)
S1	500	0	100
S2	300	40	60
S3	150	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening.

COLORIEMTRIC PROCEDURE

	Standard well	Sample well	Blank well
Each diluted Standard	10 µL		
Each Sample		10 µL	10 µL
Working Reagent	90 µL	90 µL	
Blank Working Reagent			90 µL
Tap plate to mix briefly and thoroughly. Incubate for **60 minutes** at **room temperature**.			
Read the absorbance at **O.D. 570 nm**. (525-605 nm)			

Note: If using an internal standard, samples will need three separate reactions: 1) sample plus standard, 2) sample alone and 3) sample blank. For the sample plus standard well, add 5 µL of 500 µM maltose and 10 µL of sample. For the sample and sample blank wells, add 5 µL of distilled water and 10 µL of sample.

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 0 to 50 µM maltose. Dilute maltose standard 1:10 in distilled water. If an internal standard is used, use 5 µL of 50 µM maltose. Use black flat-bottom 96 well microplate for assay. And read the fluorescence at λex/em = 530/585 nm.

CALCULATION OF RESULTS

1. Subtract blank value (distilled water, S4) from the standard values and plot the ΔOD or ΔRFU against standard concentrations. Determine the slope and calculate the maltose concentration of Sample as follows:

   Maltose (µM) = $[(R_{Sample} - R_{Blank}) / Slope\ (\mu M^{-1})] \times n$

2. If an internal standard was used, the sample maltose concentration is computed as follows:

   Maltose (µM) = $[(R_{Sample} - R_{Blank}) / (R_{Standard} - R_{Sample})] \times (Standard / 2) \times n$

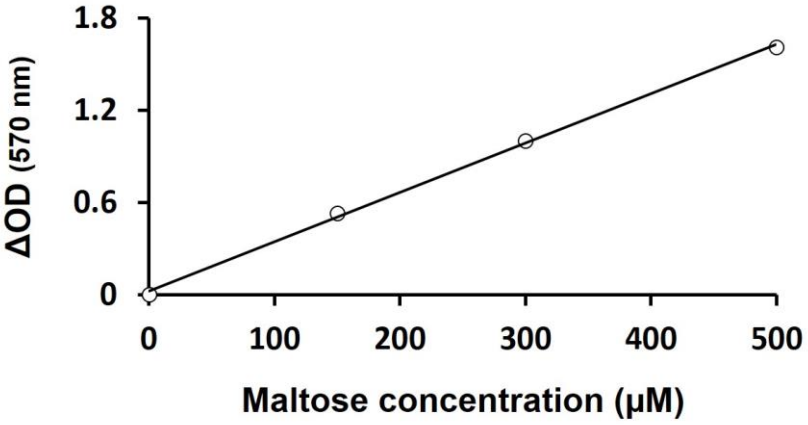
   Note:

   - R_{Sample}, R_{Blank} and $R_{Standard}$: the O.D. 570 nm values and fluorescence intensity values of the sample and sample blank and sample plus Standard respectively.
   - n is the sample dilution factor.
   - The volume of the internal standard is 2x lower than the sample volume; thus, the internal standard concentration should be divided by 2.

2. Conversions: 1 mM maltose equals 34.23 mg/dL, or 342.3 ppm.
3. If the calculated maltose concentration is >500 µM for the colorimetric assay, or >50 µM for the fluorimetric assay, dilute sample in distilled water and repeat assay. Multiply result by the dilution factor n.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Maltose Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

2 μM