

EnzyChrom™ Maltose Assay Kit (EMLT-100)

Quantitative Colorimetric/Fluorimetric Maltose Determination

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.

BioAssay Systems' maltose assay 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 at $\lambda_{ex/em} = 530/585$ nm is directly proportional to maltose concentration in the sample.

KEY FEATURES

Fast and sensitive. Use of 10 μ L sample. Linear detection range 2 to 500 μ M Maltose for colorimetric assays and 1 to 50 μ M for fluorimetric assays.

Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 60 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: Maltose in various biological samples such as serum, urine, food and beverages, etc.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	Dye Reagent:	120 μ L
Enzyme A:	120 μ L	Standard:	1 mL 5 mM Maltose
Enzyme Mix:	120 μ L		

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

Precautions: Reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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

Serum can be assayed directly, after centrifuging first to remove any particulates. Appropriate dilution in distilled water may be required.

Urine samples can be assayed directly, after centrifuging first to remove any particulates, and requires the use of an internal standard.

Colorimetric Procedure

- Standards.** Prepare 500 μ L of 500 μ M Premix by mixing 50 μ L of the Standard (5 mM) and 450 μ L distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H ₂ O	Maltose (μ M)
1	100 μ L + 0 μ L	500
2	60 μ L + 40 μ L	300
3	30 μ L + 70 μ L	150
4	0 μ L + 100 μ L	0

Transfer 10 μ L standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 10 μ L of each sample into separate wells.

- Samples.** Add 10 μ L of each sample to two separate wells in a 96 well plate (each sample requires a sample blank).

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 μ L 500 μ M maltose and 10 μ L sample. For the sample and sample blank wells, add 5 μ L dH₂O and 10 μ L sample.

- Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 95 μ L Assay Buffer, 1 μ L Enzyme A, 1 μ L Enzyme Mix, and 1 μ L Dye Reagent. For the sample blanks, prepare a Blank WR (BWR) **without** Enzyme A.

Add 90 μ L WR to the *four Standards* and the *Sample Wells* and 90 μ L BWR to the *Sample Blank Wells*. Tap plate to mix briefly and thoroughly. Incubate 60 minutes at room temperature.

- Read optical density at 570 nm (525-605 nm).

Fluorimetric Procedure

- Standards.** Dilute the standards prepared in *Colorimetric Procedure* 1:10 in dH₂O. If an internal standard is used, use 5 μ L of 50 μ M maltose.
- Transfer 10 μ L standards into separate wells of a black 96-well plate. Transfer 10 μ L of each sample into separate wells (2 wells per sample if a standard curve is used, 3 wells per sample if an internal standard is used).
- Add 90 μ L of appropriate WR (see *Colorimetric Procedure*) to each well. Tap plate to mix briefly and thoroughly. Incubate 60 minutes at room temperature.
- Read fluorescence at $\lambda_{ex/em} = 530/585$ nm.

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the maltose concentration of the Sample,

$$[\text{Maltose}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

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

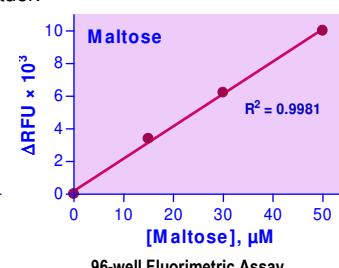
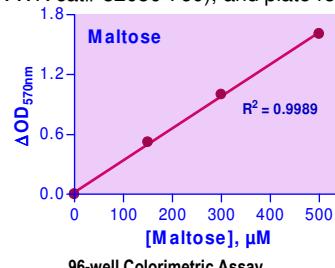
$$[\text{Maltose}] = \frac{R_{\text{SAMPLE}} - R_{\text{BLANK}}}{R_{\text{STANDARD}} - R_{\text{SAMPLE}}} \times \frac{[\text{Standard}]}{2} \times n \quad (\mu\text{M})$$

where R_{SAMPLE} , R_{BLANK} and R_{STANDARD} are OD or fluorescence readings of the Sample, Sample Blank and the Sample plus Standard respectively. n is the sample dilution factor. Note: The volume of the internal standard is 2x lower than the sample volume; thus, the internal standard concentration should be divided by 2. If the calculated maltose concentration is >500 μ M for the colorimetric assay, or >50 μ M for the fluorimetric assay, dilute sample in dH₂O and repeat assay. Multiply result by the dilution factor n .

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

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



LITERATURE

- Young, J.M., and E. Weser (1971). The Metabolism of Circulating Maltose in Men. *J Clin Invest* 50: 986-991.
- Aono, K., et al (1982). Maltose as an Energy Substrate During Surgery: Comparison of Maltose with Glucose. *Can Anaesth Soc J* 3: 236-42.
- Floridi, S., et al (2001). Carbohydrate Determination in Wort and Beer by HPLC-ELSD. *Monatsschrift für Brauwissenschaft* 54(9): 209-215.

