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Product Information

Maltose and Glucose Assay Kit

Catalog Number **MAK018**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Maltose is a disaccharide containing two glucose molecules with an α(1→4) glycosidic linkage. The enzyme α-D-Glucosidase hydrolyzes maltose to its constituent monosaccharides. Glucose is the primary carbohydrate utilized for energy during cellular respiration. Maltose can be derived from starch in food through the action of amylase. Maltose can be found in many food products, including beer, cereals, and pasta.

The Maltose and Glucose Assay kit provides a simple and direct procedure for measuring maltose and glucose in a variety of samples, including serum, plasma, food, or growth media. In this kit, maltose is converted to two glucose units via α-D-Glucosidase. Glucose is further oxidized, resulting in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the maltose present. This kit has a linear detection range of 0.1–0.5 nmole maltose for the fluorometric assay and 1–5 nmoles maltose for the colorimetric assay.

Components

The kit is sufficient for 100 assays in 96 well plates.

Component	Amount
Glucose Assay Buffer Catalog Number MAK018A	25 mL
Glucose Probe Catalog Number MAK018B	1 vl
α-D-Glucosidase Catalog Number MAK018D	1 vl
Glucose Enzyme Mix Catalog Number MAK018E	1 vl
Maltose Standard, 100 nmole/μL Catalog Number MAK018F	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

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.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Glucose Assay Buffer – Allow buffer to come to room temperature before use.

Glucose Probe – Thaw at room temperature to melt solution prior to use. Store protected from light and moisture at –20 °C. Use within 2 months. Upon thawing, the Glucose Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Glucose Probe Solution 5 to 10-fold with Glucose Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

α-D-Glucosidase and Glucose Enzyme Mix – Reconstitute each with 220 μL of Glucose Assay Buffer. Mix well by pipetting (don't vortex), then aliquot each and store, protected from light and moisture, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Maltose Standards for Colorimetric Detection
Dilute 5 μL of the 100 nmole/μL Maltose Standard with 995 μL of the Assay Buffer to prepare a 0.5 nmole/μL standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 0.5 nmole/μL standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 μL.

Maltose Standards for Fluorometric Detection
Prepare a 0.5 nmole/μL solution as for the colorimetric assay. Dilute 20 μL of the 0.5 nmole/μL solution with 180 μL of the Glucose Assay Buffer to prepare a 0.05 nmole/μL solution. Add 0, 2, 4, 6, 8, and 10 μL of the 0.05 nmole/μL standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 μL.

Sample Preparation
Samples may be assayed directly. Serum may diluted directly with Glucose Assay Buffer. Add up to 50 μL of sample to wells. Bring samples to a final volume of 50 μL with Glucose Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
Note: To detect maltose, prepare two wells for each sample. Add 2 μL of α-D-Glucosidase to one well to convert maltose to glucose (total glucose detection). Do not add α-D-Glucosidase to the other well (free glucose detection).

1. Set up Master Reaction Mix according to the scheme in Table 1. 50 μL of the MasterReaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Volume**
Glucose Assay Buffer	46 μL
Glucose Probe	2 μL
Glucose Enzyme Mix	2 μL

2. Add 50 μL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 37 °C. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) maltose standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate maltose standards to plot a standard curve. The amount of glucose present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Maltose and Glucose

Total Glucose: $C_T = S_a/S_v$ with α-D-Glucosidase
Free Glucose: $C_F = S_a/S_v$ without α-D-Glucosidase
Maltose = (Total Glucose – Free Glucose)/2

S_a = Amount of glucose in unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added to reaction well
C = Concentration of glucose in sample

Maltose molecular weight: 342.3 g/mole
Glucose molecular weight: 180.2 g/mole

Sample Calculation

Amount of total glucose (S_a) = 3.84 nmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of total glucose in sample

$$3.84 \text{ nmole}/50\ \mu\text{L} = 0.0768 \text{ nmole}/\mu\text{L}$$

Amount of free glucose (S_a) = 1.84 nmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of free glucose in sample

$$1.84 \text{ nmole}/50\ \mu\text{L} = 0.0368 \text{ nmole}/\mu\text{L}$$

Concentration of maltose in sample

$$\frac{0.0768 - 0.0368}{2} = 0.020 \text{ nmole}/\mu\text{L}$$

$$0.020 \text{ nmole}/\mu\text{L} \times 342.3 \text{ ng/nmole} = 6.85 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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