Maltose Assay Kit

(Catalog #K628-100; 100 assays; Store at -20˚C)

I. 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.).

II. Kit Contents

Component	K628-100	Cap Code	Part No.
Maltose Assay Buffer	25 ml	WM	K628-100-1
Maltose Probe (in DMSO, Anhydrous)	0.2 ml	Red	K628-100-2A
α-D-Glucosidase (Lyophilized)	1 Vial	Blue	K628-100-4
Enzyme Mix (Lyophilized)	1 Vial	Green	K628-100-5
Maltose Standard (100 nmol/µl)	100 µl	Yellow	K628-100-6

III. Storage and Handling:

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

IV. Reagent Preparation and Storage Conditions:

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.

V. Assay Protocol:

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 maltose to glucose:** Add 2 µl of Glucosidase* into each standard and sample well, mix well.
   ***Notes:** 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 sample amount from maltose standard curve.
Sv is the sample volume added in sample wells.
Maltose molecular weight: 342.3; Glucose: 180.2.

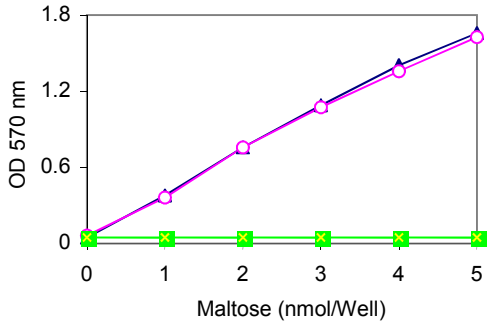

Figure Legend: Maltose Standard Curve. Assays were performed following kit instructions. Open square is maltose with α-D-Glucosidase. Solid square is maltose without α-D-Glucosidase. Triangle is free glucose (divided by 2).

FOR RESEARCH USE ONLY! Not to be used on humans.

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• 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 tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		