Maltose and Glucose Assay Kit

(Catalog #K618-100; 100 assays; Store at -20°C)

I. Introduction:

Glucose ($C_6H_{12}O_6$; FW: 180.16) and Maltose ($C_{12}H_{22}O_{11}$; FW: 342.3) are the main fuel sources to generate the universal energy molecule ATP. Maltose is the major disaccharide 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 molecules of glucose by α-D-glucosidase. Glucose oxidase specifically oxidizes free glucose to produce a product that interacts with the glucose probe to generate color and fluorescence. Therefore, glucose or maltose levels in various biological samples (e.g. serum, plasma, body fluids, food, growth medium, etc.) can be easily determined by either colorimetric (spectrophotometry at λ = 570 nm) or fluorometric (Ex/Em = 535/587 nm) methods. The assay can detect 10 pmol to 10 nmol glucose per assay.

II. Kit Contents

Component	K618-100	Cap Code	Part No.
Glucose Assay Buffer	25 ml	WM	K618-100-1
Glucose Probe (in DMSO)	0.2 ml	Red	K618-100-2A
α-D-Glucosidase (Lyophilized)	1 Vial	Blue	K618-100-4
Glucose Enzyme Mix (Lyophilized)	1 Vial	Green	K618-100-5
Maltose Standard (100 nmol/µl)	100 µl	Yellow	K618-100-6

III. Storage and Handling:

Store kit at –20°C, protect from light. Allow reagents warm to room temperature and briefly centrifuge vials before opening.

IV. Reagent Preparation:

Glucose Probe: Ready to use as supplied. Warm the vial to room temperature to thaw the DMSO solution before using. Store at –20°C, protect from light. Use within two months.

α-D-Glucosidase: Dissolve in 220 µl Glucose Assay Buffer by pipetting up and down. Aliquot and store at –20°C. Use within two months.

Glucose Enzyme Mix: Dissolve in 220 µl Glucose Assay Buffer 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 Maltose Standard to 0.5 nmol/µl by adding 5 µl of the Maltose Standard to 995 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96-well plate. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 1, 2, 3, 4, 5 nmol/well of Maltose Standard.

For the fluorometric assay, dilute the Maltose Standard solution to 0.05 nmol/µl by adding 5 µl of the Maltose Standard to 995 µl of Glucose Assay Buffer, mix well. Then take 20 µl into 180 µl of Glucose Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96-well plate. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of the Maltose Standard. If a more sensitive assay is desired, the Maltose standard can be further diluted 10 fold more, and then follow the same procedure.

2. **Sample Preparation:** Prepare test samples in 50 µl/well with Glucose Assay Buffer in a 96-well plate. Serum can be directly diluted in the Glucose Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve linear range.

   If you want to specifically detect maltose, prepare two wells for each sample. Add 2 µl of α-D-Glucosidase to one well to convert maltose to glucose to detect total glucose. Omit α-D-Glucosidase in the other well to detect free glucose. Then, Maltose = (Total Glucose – Free Glucose)/2.

3. **Glucose 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 Glucose Assay Buffer
   2 µl Glucose Probe
   2 µl Glucose Enzyme Mix

4. Mix well. Add 50 µl of the Reaction Mix to each well containing the Maltose Standard and test samples. Mix well.

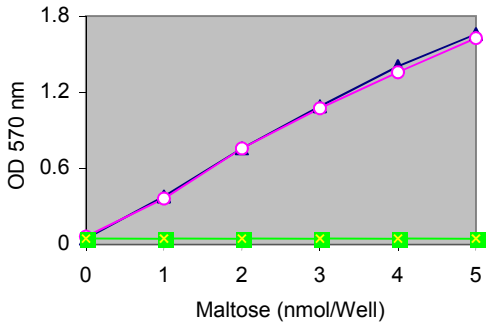
5. Incubate the reaction for 60 min at 37°C, protect from light.

6. Measure OD 570nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.

7. 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). Plot the standard curve. Apply sample readings to the standard curve. The concentration can then be calculated:

   Free Glucose = 2As / Sv without addition of α-D-Glucosidase
   Total Glucose = 2As / Sv with addition of α-D-Glucosidase
   Maltose = (Total Glucose – Free Glucose)/2

   Where: As is Glucose amount from maltose standard curve.
   Sv is the sample volume added in sample wells.
   Glucose molecular weight: 180.2.; Maltose: 342.3.



Maltose Standard Curve: Assays were performed using various amounts of Maltose standard according to kit instructions. Open square is maltose with α-D-Glucosidase. Solid square is maltose without α-D-Glucosidase. Triangle is glucose with 2 times nmol of Maltose.

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

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.		