Product Information

Maltose Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2132-100 100 assays	Cap Color	Part Number
Maltose Assay Buffer	25 ml	WM	K2132-C-1
Maltose Probe (in DMSO, Anhydrous)	0.2 ml	Red	K2132-C-2
α-D-Glucosidase (Lyophilized)	1 Vial	Blue	K2132-C-3
Enzyme Mix (Lyophilized)	1 Vial	Green	K2132-C-4
Maltose Standard (100 nmol/µl)	100 µl	Yellow	K2132-C-5

II. Introduction:

Maltose ($C_{12}H_{22}O_{11}$) is a major disaccharide formed from two units of glucose joined by an α-1,4-glycosidic linkage and is produced from hydrolysis of starch in food. Maltose is a main fuel source to generate the energy molecule ATP. Maltose can be easily converted to two glucoses by α-D-glucosidase.

The Maltose Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for detection of maltose in various biological samples (plasma, serum, body fluids, growth medium, food, etc.) based on colorimetric and fluorometric method. In the assay, maltose is converted to glucose by α-D-glucosidase. Glucose is specifically oxidized to generate a product that interacts with the probe to yield fluorescence (Ex/Em = 538/587 nm) and color (λmax = 570 nm).

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:

Assay Buffer	46 µl
Probe	2 µl
Enzyme Mix	2 µl

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.

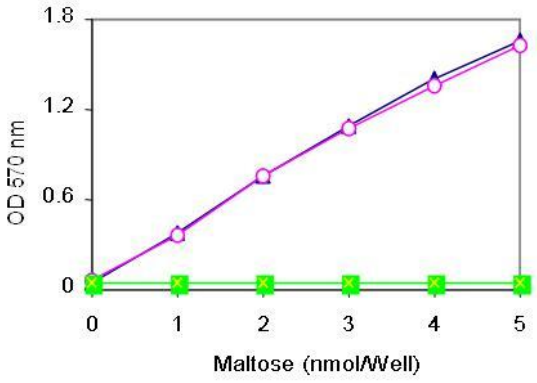
8. Calculations: Correct background by subtracting the value derived from the 0 maltose standard from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Subtract glucose background from maltose samples. Plot the standard curve. Apply sample readings to the standard curve. The concentration can then be calculated:

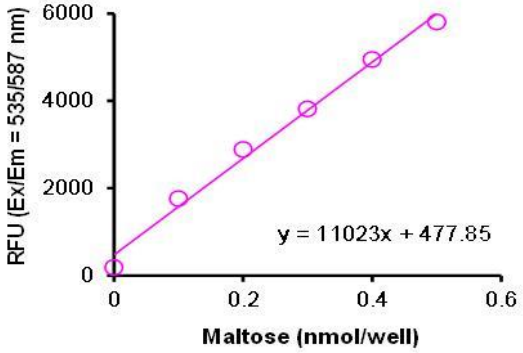
C = Sa/Sv nmol/ml, or µmol/ml, or mM

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.





General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings

	• Use of a different 96-well plate	of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com