ab65335

Maltose and Glucose Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Maltose and Glucose levels in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version: 4 Last Updated: 7 September 2023

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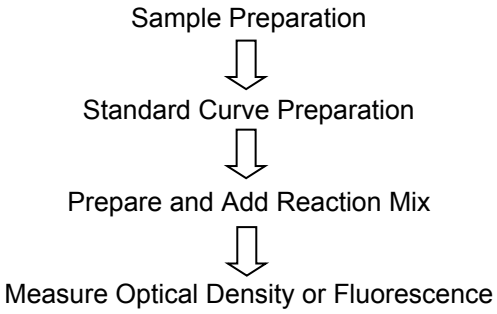
1. Overview

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 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. Glucose oxidase specifically oxidizes free glucose to produce a product that interacts with the OxiRed Probe/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. Abcam's Maltose and Glucose Assay Kit can detect 10 pmol to 10 nmol glucose per assay.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer II/Glucose Assay Buffer	25 mL
OxiRed Probe/Glucose Probe (in DMSO)	0.2 mL
α-D-Glucosidase (Lyophilized)	1 vial
Development Enzyme Mix II/Glucose Enzyme Mix (Lyophilized)	1 vial
Maltose Standard (100 nmol/μl)	100 µL

* Store kit at -20°C, protect from light. Allow reagents to warm to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

OxiRed Probe/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 AND Development Enzyme Mix II/Glucose GLUCOSE ENZYME MIX : Dissolve separately in 220 µl Glucose Assay Buffer II/Assay Buffer. Aliquot and store at -20°C. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Prepare test samples in 50 µl/well with Assay Buffer II/Glucose Assay Buffer in a 96-well plate. Serum can be directly diluted in the Assay Buffer II/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 in one well to convert maltose to glucose to detect total glucose. Use the other well for detecting free glucose without the addition of α-D-Glucosidase.

$$\text{Then, } \textbf{Maltose} = \frac{\textbf{(Total Glucose – Free Glucose)}}{\textbf{2}}$$

2. Standard Curve Preparation:

a. 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 Assay Buffer II/Glucose 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 II/Glucose Assay Buffer to generate 0, 1, 2, 3, 4, 5 nmol/well of Maltose Standard.

b. 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 Assay Buffer II/Glucose Assay Buffer, mix well. Then take 20 µl into 180 µl of Assay Buffer II/Glucose 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 II/Glucose Assay Buffer to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of the Maltose Standard.

Note:

If a more sensitive assay is desired, the Maltose standard can be further diluted 10 fold more, and then follow the same procedure.

3. Glucose Reaction Mix: Mix enough reagent for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

Assay Buffer II/Glucose Assay Buffer	46 µl
Development Enzyme Mix II/ Glucose Glucose Enzyme Mix	2 µl
OxiRed Probe/Glucose Probe	2 µl

The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the OxiRed Probe/probe per reaction to decrease the background reading/increase detection sensitivity significantly.

Mix well. Add 50 µl of the Reaction Mix to each well containing the Maltose Standard and test samples, mix well. Incubate the reaction for 60 min at 37°C, protect from light.

4. Measurement: Measure OD_{570nm} for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.

5. Data Analysis

Correct background by subtracting the value derived from the zero maltose standard from all sample readings. 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

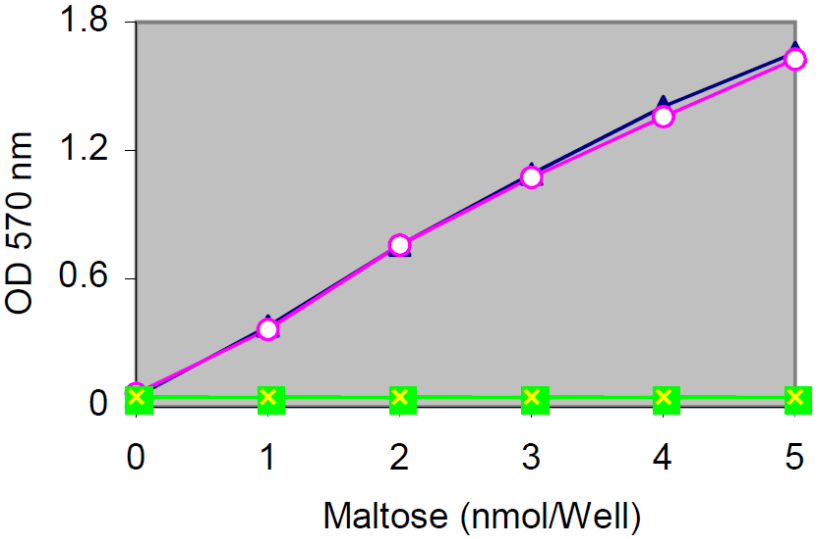
$$\textbf{Maltose} = \frac{\textbf{(Total Glucose – Free Glucose)}}{\textbf{2}}$$

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 circle is maltose with α-D-Glucosidase. Solid square is maltose

without α-D-Glucosidase. Triangle is glucose with 2 times nmol of Maltose.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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