ab83388

Maltose Assay Kit

Instructions for Use

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

View kit datasheet: www.abcam.com/ab83388
(use www.abcam.cn/ab83388 for China, or www.abcam.co.jp/ab83388 for Japan)

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: 2 Last Updated: 24 May 2013

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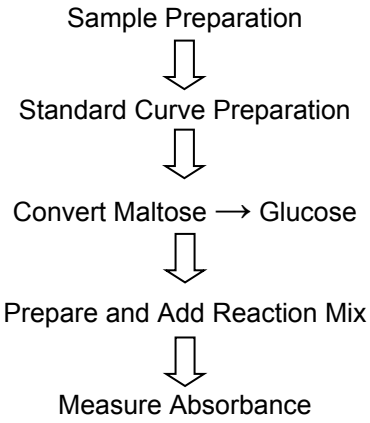
1. Overview

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 maltose units joined by a α-1,4-glycosidic linkage, which can be easily converted to two maltoses by α-D-glucosidase. The generated maltose 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.

Abcam's Maltose Assay 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.).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer II/Maltose Assay Buffer	25 mL
OxiRed Probe/Maltose Probe (in DMSO)	0.2 mL
α-D-Glucosidase (Lyophilized)	1 vial
Development Enzyme Mix II/Maltose 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/MALTOSE PROBE: Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at -20°C, protect from light. Use within two months.

α-D-GLUCOSIDASE AND Development Enzyme Mix II/MALTOSE ENZYME MIX : Dissolve separately in 220 µl Assay Buffer II/Maltose Assay Buffer. Pipette up and down. 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/Maltose Assay Buffer in a 96-well plate. Serum can be directly diluted in the Assay Buffer II/Maltose Assay Buffer.

For unknown samples we suggest testing several doses of your sample to make sure the readings are within the standard curve linear range.

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

Note:
The fluorometric assay is ~10 times more sensitive than the colorimetric assay.

3. Convert Maltose to Glucose: Add 2 µl of α-D-Glucosidase***** into each standard and sample well, mix well.

*** Note:** 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 α-D-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 II/assay buffer into the other well as glucose background control.

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

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.

****Note:** In the fluorometric assay, using 0.4 µl OxiRed Probe/probe for each reaction will significantly decrease fluorescence background, and thus increase fluorescence signal/noise ratio.

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:

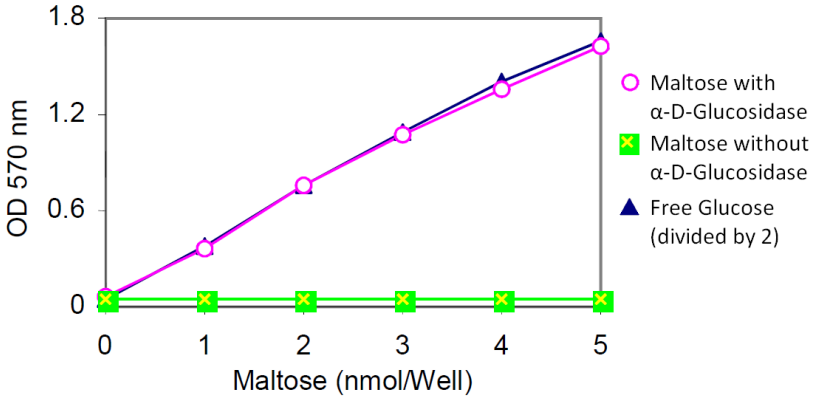
Concentration = Sa / Sv (nmol/ml, or µmol/ml, or mM)

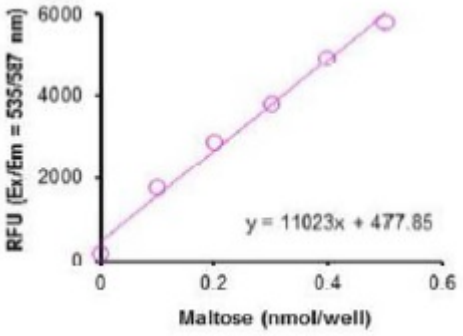
Where:

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.


1.8
1.2
0.6
0
OD 570 nm
0
1
2
3
4
5
Maltose (nmol/Well)
Maltose with α-D-Glucosidase
Maltose without α-D-Glucosidase
Free Glucose (divided by 2)


6000
4000
2000
0
RFU (Ex/Em = 535/587 nm)
y = 11023x + 477.85
0
0.2
0.4
0.6
Maltose (nmol/well)

Maltose Standard Curve. Assays were performed following kit instructions.

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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