RayBio® Maltose and Glucose Assay Kit

User Manual Version 1.0
May 8, 2014

RayBio® Maltose and Glucose Assay Kit Protocol

(Cat#:68-MalGlu-S100)

RayBiotech, Inc.

We Provide You With Excellent Support And Service

Tel:(Toll Free)1-888-494-8555 or 770-729-2992; Fax:770-206-2393;
Web: www.raybiotech.com Email: info@raybiotech.com

RayBiotech, Inc.

RayBio® Maltose and Glucose Assay Kit

TABLE OF CONTENTS

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

Components	Size	Cap Code	Part Number
Glucose Assay Buffer	25 ml	WM	Item A
Glucose Prove (in DMSO)	0.2 ml	Red	Item B
α-D-Glucosidase (Lyophilized)	1 vial	Blue	Item C
Glucose Enzyme Mix (Lyophilized)	1 vial	Green	Item D
Maltose Standard (100 nmol/µl)	100 µl	Yellow	Item E

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 37C, 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:

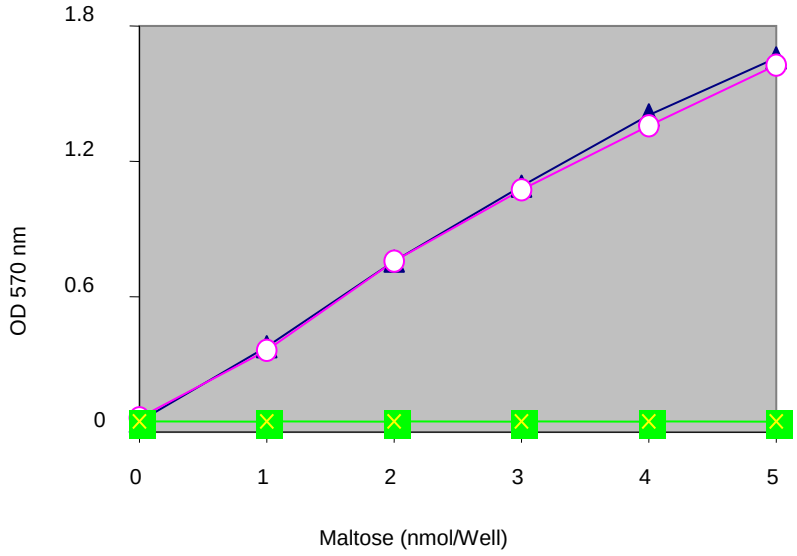
Free Glucose = 2As / Sv without addition of α-D-Glucosidase

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

VI. GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• 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 of the instrument • Fluorescence: Black plates (clear bottoms) ; 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 datasheet) • 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 datasheet & 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.		

This product is for research use only.