Version 1 Last updated 5 June 2020

ab273317 Maltose Phosphorylase Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273317
(use https://www.abcam.cn/ab273317 for china, or
https://www.abcam.co.jp/ab273317 for Japan)

For the determination of Maltose Phosphorylase activity in different bacterial lysates.

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

Table of Contents

1. Overview

Maltose Phosphorylase Assay Kit (Fluorometric) (ab273317) is the first commercial assay to determine the Maltose Phosphorylase activity in different bacterial lysates. The assay converts maltose to produce Glucose, which is then detected by a set of enzymatic reactions to generate a fluorescent product with a Ex/Em 535/587 nm. The fluorescence signal is directly proportional to the Maltose Phosphorylase activity.

This Kit is rapid, sensitive and a convenient tool for detecting Maltose Phosphorylase activity. It can detect as low as 0.5 mU under the assay conditions.

2. Protocol Summary

Prepare samples as directed

Prepare all reagents as directed

Prepare standard curve and measure fluorescence at Ex/Em = 535/587 nm. Calculate slope

Add Positive Control, Samples, Background Control to appropriate wells and adjust volume to 50 µL

Add Reaction Mix or Background Mix(50 µL)

Measure fluorescence (Ex/Em= 535/587 nm) in a kinetic mode for 30 min at 25°C.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
Maltose Assay Buffer	25 mL	4°C
Maltose Probe	200 µL	-20°C
Maltose Developer (Lyophilized)	1 vial	-20°C
Maltose (Lyophilized)	1 vial	-20°C
Glucose Standard	100 µL	-20°C
Maltose Phosphorylase	50 µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Sonicator
- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96-well black plate with flat bottom
- 50% glycerol
- PBS

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Maltose Assay Buffer:**

Ready to use. Warm bottle to room temperature.

Store at 4°C.

9.2 **Maltose Probe (in DMSO):**

Ready to use as supplied. Warm to RT prior to use to melt frozen DMSO.

Store at -20°C, protected from light and moisture.
Use within two months.

9.3 **Maltose Developer:**

Reconstitute with 220 µl of Assay Buffer.

Keep on ice while in use.
Store at -20°C.
Use within two months.

9.4 **Maltose:**

Reconstitute with 220 µl of Assay Buffer.

Keep on ice while in use.
Store at -20°C.
Use within two months.

9.5 **Glucose Standard:**

Store at -20°C. Warm to RT before use.

9.6 **Maltose Phosphorylase:**

Add 450 µl of 50% glycerol (not included) to the vial to prepare the enzyme stock. Vortex to mix.
Aliquot and store at -20°C.
Avoid multiple freeze-thaw cycles.
Use within two months.

10. Sample Preparation

10.1 Cells preparation:

10.1.1 Grow cells in a suitable growth medium at 37°C overnight.
10.1.2 After incubation, centrifuge at 10,000 x g for 20 min to harvest the cells.
10.1.3 Measure the weight of the pellet.
10.1.4 Add 5 ml of ice-cold PBS into the cell pellet and disperse the pellet.
10.1.5 Sonicate the cells for 5-10 min on ice.
10.1.6 After sonication, centrifuge the cells at 10,000 x g for 30 min
10.1.7 Transfer the supernatant to a new tube.

Δ Note: For Unknown Samples, we recommend doing a pilot experiment and testing several doses to ensure the readings are within the linear range of the Standard Curve.

10.2 Positive Control:

10.2.1 Prepare a 10-fold dilution of the Maltose Phosphorylase enzyme by adding 5 µl of the enzyme with 45 µl of Maltose Assay Buffer.

11. Standard Curve

11.1 Prepare 1 mM diluted Glucose Standard solution by adding 10 µl of Glucose Standard with 990 µl of Assay Buffer.

11.2 Prepare a 0.1 mM diluted Glucose Standard solution by adding 100 µl of 1 mM diluted Glucose Standard with 900 µl of Assay Buffer.

11.3 Add 0, 4, 8, 12, 16 and 20 µl of the 0.1 mM diluted Standard into the desired wells and adjust the volume to 50 µl using Maltose Assay Buffer. This will generate 0, 0.4, 0.8, 1.2, 1.6 and 2.0 nmole of Standard/well respectively.

Standard #	0.1 mM Glucose Standard (µL)	Assay Buffer (µL)	Glucose (nmol/well)
1	0	50	0
2	4	44	0.4
3	8	42	0.8
4	12	38	1.2
5	16	34	1.6
6	20	30	2.0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1 Add 2 µl of Sample, Background and Positive Control to appropriate wells a 96-well black flat-bottom plate.

12.1.1 Adjust the volume to 50 µl using Maltose Assay Buffer.

Reaction Mix:

12.1.2 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of each Mix:

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer	45.5	47.5
Maltose	2	0
Maltose Developer	2	2
Maltose Probe	0.5	0.5

12.1.3 Add 50 µl of Reaction Mix to each wells containing Standard, Sample(s) or Positive Control and mix well.

12.1.4 For Sample Background Control, add 50 µl of Background Control mix to each well, mix well..

12.1.5 Measure the RFU signal in a kinetic mode at 25°C for 30 min.

12.1.6 After the reaction completes, the RFU signal may start to decrease. Therefore, use the maximum RFU at Ex/Em = 535/587 nm for calculation.

13. Calculations

13.1 Subtract the 0 Standard reading from all Standard readings.
13.2 Plot the Glucose Standard Curve.
13.3 Choose two time points within the linear portion of the curve (t1 and t2) for each Sample.

Δ Note: If the Sample Background Control reading is significant, subtract the Sample Background reading from all Sample readings to get the corrected Sample readings.

13.4 Apply the corrected Sample readings to the Standard Curve to get A nmol of Glucose formed during the reaction time (Δt=t2-t1).
13.5 Calculate the Maltose Phosphorylase activity of the Samples:

$$Specific\ activity\ = \frac{A\ x\ D}{(\Delta t\ x\ M)}\ \ (mU/mg)$$

A = Glucose from the Standard Curve
Δt = Reaction time (min)
M = Sample used (in mg)
D = Dilution factor (D = 1 for undiluted Samples)

Unit definition:

One unit is 1 µmole of Glucose generated per min at pH 7 and 25°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

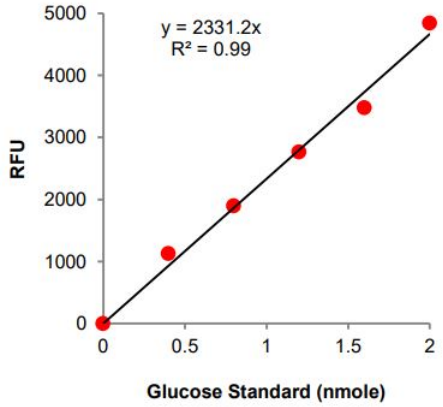

Figure 1. Glucose Standard Curve.

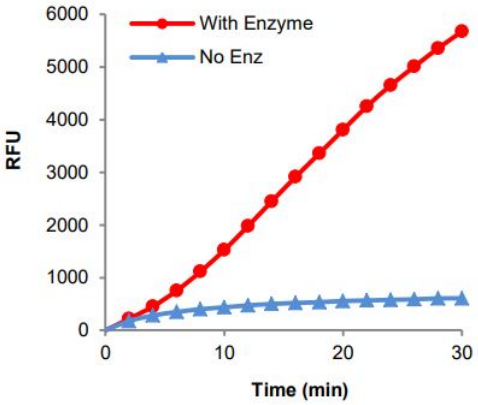

Figure 2. Reaction curve for Maltose Phosphorylase activity.

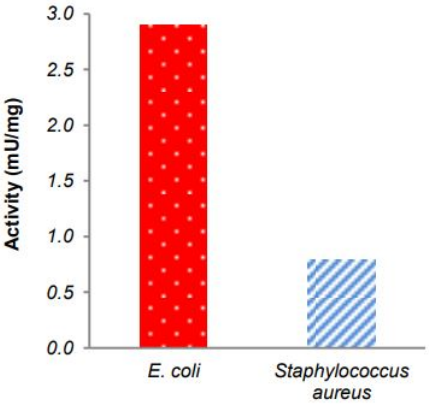

Figure 3. Maltose Phosphorylase activity using E. coli and Staphylococcus aureus cell lysates.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)