

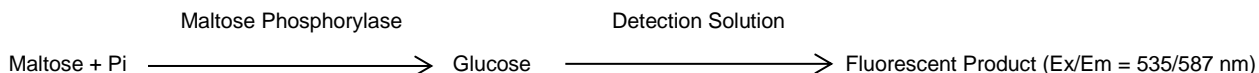
Maltose Phosphorylase Activity Kit (Fluorometric)

6/19

(Catalog # K353-100; 100 assays, Store kit at -20°C)

I. Introduction:

Maltose phosphorylase (EC 2.4.1.8) belongs to the family of hexosyltransferases. It catalyzes maltose and inorganic phosphate to produce glucose and glucose-1-phosphate (G1P) during Maltose metabolism. Maltose phosphorylase is commonly identified in gram positive and gram negative bacteria including *E. coli*, *Bacillus subtilis*, *Enterococcus sp.* and *Lactobacillus brevis* etc. BioVision's Maltose Phosphorylase Activity Assay Kit 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. BioVision's Maltose Phosphorylase Activity Assay 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.



II. Application:

- Measurement of Maltose Phosphorylase activity in different bacteria
- Analysis of Maltose Phosphorylase kinetics in inhibition or activation screening

III. Sample Type:

- Bacterial lysates

IV. Kit Contents:

Components	K353-100	Cap Code	Part Number
Maltose Assay Buffer	25 ml	WM	K353-100-1
Maltose Probe	200 µl	Red	K353-100-2
Maltose Developer	1 vial	Green	K353-100-3
Maltose	1 vial	Orange	K353-100-4
Glucose Standard	100 µl	Yellow	K353-100-5
Maltose Phosphorylase	50 µl	Blue	K353-100-6

V. User Supplied Reagents and Equipment:

- Sonicator
- 96-well black flat-bottom plate
- Multi-well spectrophotometer
- 50% glycerol
- PBS

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **Maltose Assay Buffer:** Ready to use as supplied. Warm bottle to room temperature (RT) before use. Store at 4°C.
- **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.
- **Maltose Developer and Maltose:** Reconstitute each component with 220 µl of Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.
- **Glucose Standard:** Store at -20°C. Warm to RT before use.
- **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.

VII. Maltose Phosphorylase Activity Assay Protocol:

1. Sample Preparation: Grow cells in a suitable growth medium at 37°C overnight. After incubation, centrifuge at 10,000 x g for 20 min to harvest the cells and measure the weight of the pellet. Add 5 ml of ice-cold PBS into the cell pellet and disperse the pellet. Sonicate the cells for 5-10 min on ice. After sonication, centrifuge the cells at 10,000 x g for 30 min and transfer the supernatant to a new tube. For Sample and Background wells, add 2 µl of the cell supernatant to the desired well(s) in a 96-well black flat-bottom plate. Adjust the volume to 50 µl using Maltose Assay Buffer.

For Positive Control, prepare a 10 fold dilution of the Maltose Phosphorylase enzyme by adding 5 µl of the enzyme with 45 µl of Maltose Assay Buffer. Add 2 µl of the diluted enzyme into the Positive Control well. Adjust the volume to 50 µl using Maltose Assay Buffer.

2. Standard Curve Preparation: Mix 10 µl of Glucose Standard with 990 µl of Assay Buffer to prepare 1 mM diluted Glucose Standard. Mix 100 µl of 1 mM diluted Glucose Standard with 900 µl of Assay Buffer to prepare 0.1 mM diluted Glucose Standard solution. Add 0, 4, 8, 12, 16 and 20 µl of the 0.1 mM diluted Standard into the desired wells to generate 0, 0.4, 0.8, 1.2, 1.6 and 2.0 nmole of Standard/well respectively. Adjust the volume to 50 µl using Maltose Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. Prepare 50 μ l of Reaction Mix and 50 μ l of Background Control Mix as indicated in the table below:

	<u>Reaction Mix</u>	<u>*Background Control Mix</u>
Maltose Assay Buffer	45.5 μ l	47.5 μ l
Maltose	2 μ l	--
Maltose Developer	2 μ l	2 μ l
Maltose Probe	0.5 μ l	0.5 μ l

Add 50 μ l of Reaction Mix to each wells containing Standard, Sample(s) or Positive Control and mix well. *For Sample Background Control, add 50 μ l of Background Control mix to each well, mix well.

Note:

a) 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.

4. Measurement: Measure the RFU signal in a kinetic mode at 25°C for 30 min. After the reaction completes, the RFU signal may start to decrease. Therefore, use the maximum RFU at Ex/Em = 535/587 nm for calculation.

5. Calculation: Subtract the 0 Standard reading from all Standard readings. Plot the Glucose Standard Curve. Choose two time points within the linear portion of the curve (t_1 and t_2) for each Sample. If the Sample Background Control reading is significant, subtract the Sample Background reading from all Sample readings to get the corrected Sample readings. Apply the corrected Sample readings to the Standard Curve to get A nmol of Glucose formed during the reaction time ($\Delta t = t_2 - t_1$). Calculate the Maltose Phosphorylase activity of the Samples:

$$\text{Specific Activity (mU/mg)} = A \times D / (\Delta t \times M)$$

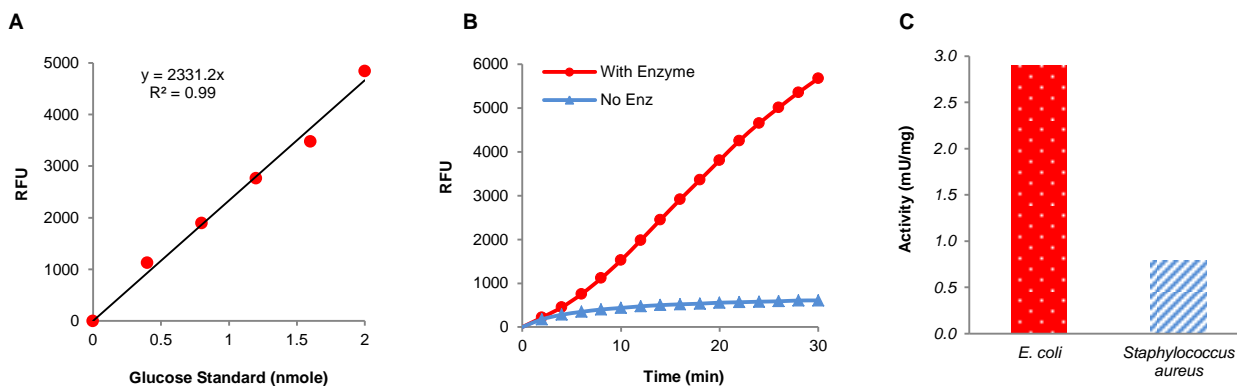
Where: **A** = Glucose from Standard Curve (nmol)

Δt = Reaction time (min)

D = Sample dilution factor

M = Sample used (in mg)

Unit Definition: One unit is 1 μ mol of glucose generated per min at pH 7 and 25°C.



Figures. A. Glucose Standard Curve. **B.** Reaction curve for Maltose Phosphorylase activity. **C.** Maltose Phosphorylase activity using *E. coli* and *Staphylococcus aureus* cell lysates.

VIII. Related Products:

α -Amylase Inhibitor Screening Kit (K482)

Maltose and Glucose Colorimetric/Fluorometric Assay Kit (K618)

Sucrose Colorimetric/Fluorometric Assay Kit (K626)

Maltose Colorimetric/Fluorometric Assay Kit (K628)

Maltose Binding Protein - Tag (MBP-Tag) Antibody (Clone 17D07) (6715)

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