# Maltose Phosphorylase (MPL-EP)

from recombinant *E. coli*

Maltose : orthophosphate 1-β-D-glucosyltransferase, EC 2.4.1.8

Maltose + Orthophosphate ⟶ D-Glucose + β-D-Glucose 1-phosphate

## SPECIFICATION

| | | |
|---|---|---|
| Appearance | white lyophilizate | |
| Activity | ≧10 U/mg lyophilizate | |
| Contaminants | α-amylase | $\leqq 5.0\times10^{-3}$% |
| | α-glucosidase | $\leqq 5.0\times10^{-2}$% |
| | NADPH oxidase | $\leqq 5.0\times10^{-2}$% |
| Stabilizer | lactose | |
| Storage | at −20°C | |

## PROPERTIES

| | | |
|---|---|---|
| Molecular weight | *ca*. 220 kDa (gel filtration) | |
| Structure | 2 subunits of 90 kDa (SDS-PAGE) | |
| Michaelis constants | $1.9\times10^{-3}$ M (maltose) | |
| | $3.4\times10^{-3}$ M (phosphate) | |
| | $8.3\times10^{-3}$ M (arsenate) | |
| pH Optimum | 6.5–7.5 | (Fig. 1) |
| pH Stability | 5.5–8.0 | (Fig. 2) |
| Optimum temperature | 45–50°C | (Fig. 3) |
| Thermal stability | below 55°C | (Fig. 4) |
| Stability (liquid form) | stable at 37°C for at least one week | (Fig. 5) |
| Stability (powder form) | stable at 30°C for at least four weeks | (Fig. 6) |
| Inhibitors | $Hg^{2+}$, $Ag^{+}$, $Zn^{2+}$, $Cu^{2+}$ | |

## ASSAY PROCEDURE

### Principle

$$\text{Maltose} + \text{Phosphate} \xrightarrow{\text{maltose phosphorylase}} \text{D-Glucose} + \beta\text{-D-Glucose-1-phosphate}$$

The appearance of D-glucose is measured spectrophotometrically at 505 nm.

### Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 $\mu$mol of D-glucose per min at 30°C and pH 7.0 under the conditions described below.

### Reagents

A. HEPES–NaOH buffer, 50 mM; pH 7.0: dissolve 2.38 g of HEPES in 160 ml of distilled water, adjust to pH 7.0 with 1 N NaOH and dilute with distilled water to 200 ml.
B. Maltose solution, 0.2 M: 3.60 g of maltose monohydrate/50 ml of HEPES–NaOH buffer (Reagent A).
C. Phosphate solution, 0.2 M: dissolve 1.36 g of $KH_2PO_4$ in 40 ml of HEPES–NaOH buffer (Reagent A), adjust to pH 7.0 with 4 N NaOH and dilute with Reagent A to 50 ml.
D. HCl solution, 5 N: 43 ml of concentrated HCl/100 ml of distilled water.
E. pH Adjusting solution (NaOH solution, 1 N): 4.0 g of NaOH/100 ml of distilled water.
F. Glucose assay kit: "GLUCOSE C II-TEST$_{WAKO}$" (Wako Pure Chemical), or a similar glucose assay kit.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.15–0.55 U/ml with ice-cold HEPES–NaOH buffer (Reagent A) immediately before measurement.

### Procedure

1. Pipette the following reagents into a test tube.
   0.2 ml HEPES–NaOH buffer (Reagent A)
   0.1 ml Maltose solution (Reagent B)
   0.1 ml Phosphate solution (Reagent C)
2. Equilibrate at 30°C for about 5 min.
3. Add 0.1 ml of sample and incubate for 10 min at 30°C [test].
   The blank solution is prepared by adding HEPES–NaOH buffer (Reagent A) instead of sample [blank].
4. Add 0.1 ml of HCl solution (Reagent D) to stop the reaction.
5. Add 0.5 ml of pH adjusting solution (Reagent E).
6. Pipette 0.3 ml of the test and blank mixture into respective test tubes.
7. Add 3.0 ml of glucose assay kit (Reagent D) and incubate for about 5 min at 37°C.
8. Read the absorbance at 505 nm in a cuvette (light path: 1 cm) [test: $A_S$, blank: $A_0$].

**Calculation**

Activity can be calculated by using the following formula:

$$\text{Volume activity (U / ml)} = \frac{(A_S - A_0) \times 1.1(\text{ml}) \times df}{6.24 \times 1/10 \times 0.1(\text{ml}) \times 10(\text{min})} = \Delta A \times 1.76 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

6.24 : Millimolar extinction coefficient of quinoneimine dye under the assay conditions ($cm^2/\mu mol$)
1/10 : A factor for correction of the reaction volume
*df* : Dilution factor
*C* : Content of maltose phosphorylase preparation in sample (mg/ml)

## APPLICATIONS

The enzyme is useful for the determination of $\alpha$-amylase and inorganic phosphate in clinical analysis.

$$\text{Maltopentaose} \xrightarrow{\alpha\text{-amylase}} \text{Maltotriose} + \text{Maltose}$$

$$\beta\text{-Maltose} \xrightarrow{\text{maltose 1-epimerase}} \alpha\text{-Maltose}$$

$$\alpha\text{-Maltose} + \text{Phosphate} \xrightarrow{\text{maltose phosphorylase}} \alpha\text{-D-Glucose} + \beta\text{-D-Glucose-1-phosphate}$$

$$\beta\text{-D-Glucose-1-phosphate} \xrightarrow{\beta\text{-phosphoglucomutase}} \beta\text{-D-Glucose-6-phosphate}$$

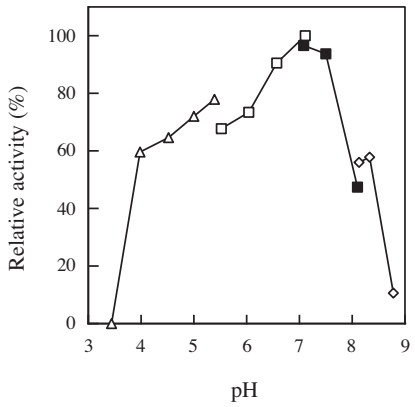
$$\text{D-Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{D-Glucono-1,5-lactone-6-phosphate} + NADPH + H^+$$

## REFERENCES

Hiruma, M. *et al*., *Nippon Nogeikagaku Kaishi*, **70**, 773–780 (1996).
Shirokane, Y. *et al.*, *Carbohydr. Res.*, **329**, 699–702 (2000).

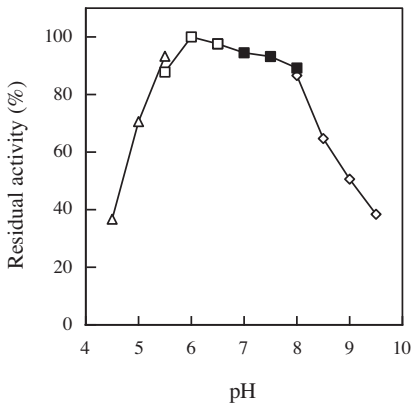
## EXPERIMENTAL DATA

Fig. 1 pH Optimum




△: 50 mM acetate buffer
□: 50 mM MES–NaOH buffer
■: 50 mM HEPES–NaOH buffer
◇: 50 mM BICINE–NaOH buffer

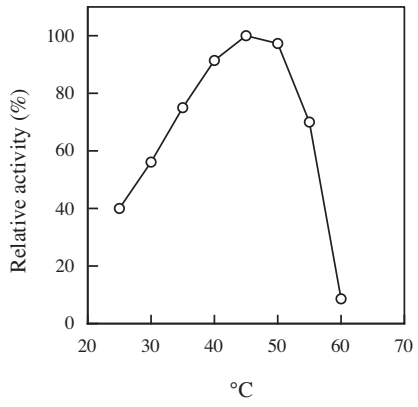
Fig. 2 pH Stability




Treatment: 37°C, 15min

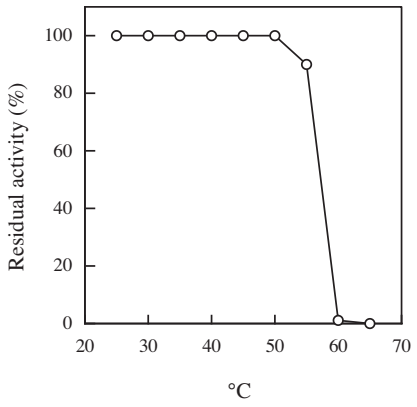
△: 50 mM acetate buffer
□: 50 mM MES–NaOH buffer
■: 50 mM HEPES–NaOH buffer
◇: 50 mM Tris–HCl buffer

Fig. 3 Optimum temperature



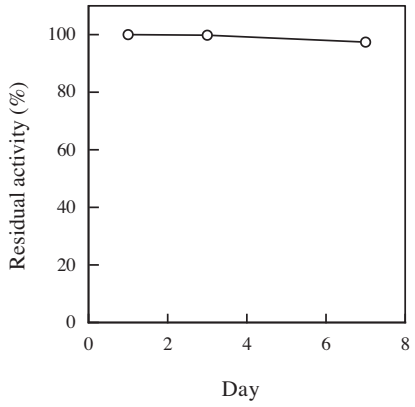

Buffer: 50 mM HEPES–NaOH buffer, pH 7.0

Fig. 4 Thermal stability



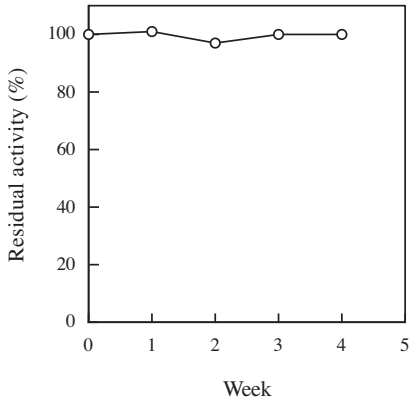

Treatment: 50 mM HEPES–NaOH buffer, pH 7.0, 10 min

Fig. 5 Stability (liquid form) at 37°C




(Kept in 50 mM HEPES–NaOH buffer, pH 7.0)

Fig. 6 Stability (powder form) at 30°C




(Kept under dry conditions)