

## Maltose Phosphorylase from *Enterococcus* sp.

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

### PROPERTIES

Molecular weight: ca. 220 kDa (gel filtration)

Structure: 2 subunits of 90 kDa (SDS-PAGE)

Michaelis constants:  $1.9 \times 10^{-3}$  M (maltose);  $3.4 \times 10^{-3}$  M (phosphate);  $8.3 \times 10^{-3}$  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)

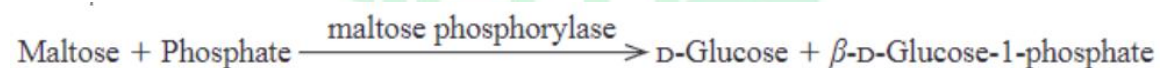
Stability (powder form): stable at 30°C for at least four weeks (Fig. 6)

Inhibitors:  $\text{Hg}^{2+}$ ,  $\text{Ag}^{+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$

STABILIZER: lactose, EDTA

### ASSAY PROCEDURE

#### Principle



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

#### 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  $\text{KH}_2\text{PO}_4$  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-TESTWAKO” (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: AS, blank: A0].

### Calculation

Activity can be calculated by using the following formula:

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

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/C$$

6.24: Millimolar extinction coefficient of quinoneimine dye under the assay conditions (cm<sup>2</sup> /μmol)

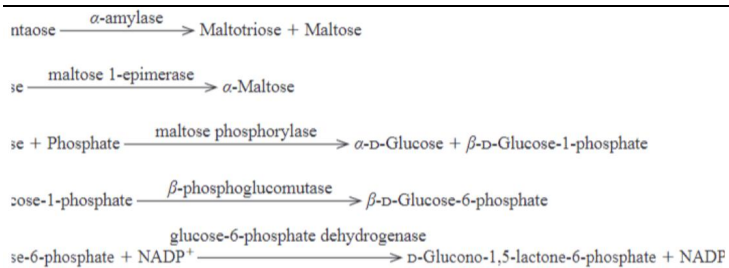
1/10: A factor for correction of the reaction volume

df: Dilution factor

C: Content of maltose phosphorylase preparation in sample (mg/ml)

### APPLICATIONS

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



## EXPERIMENTAL DATA

Fig. 1 pH Optimum

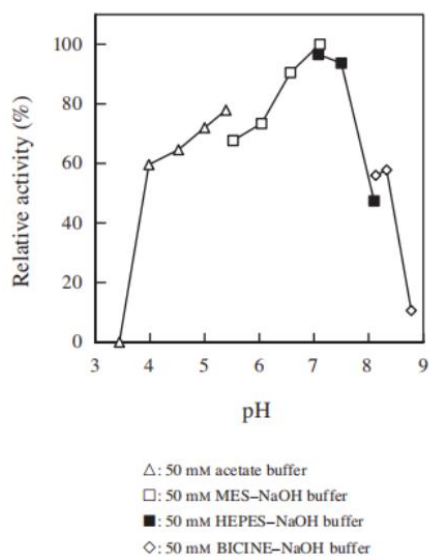


Fig. 2 pH Stability

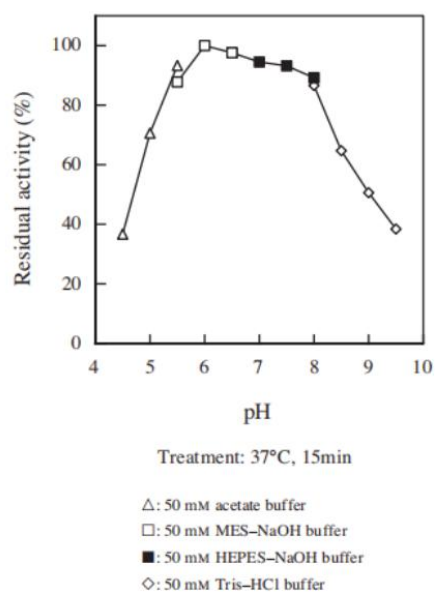


Fig. 3 Optimum temperature

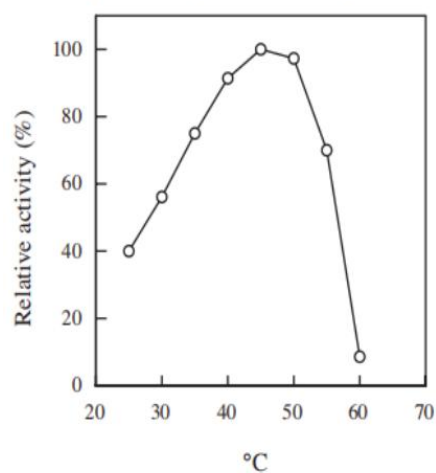


Fig. 4 Thermal stability

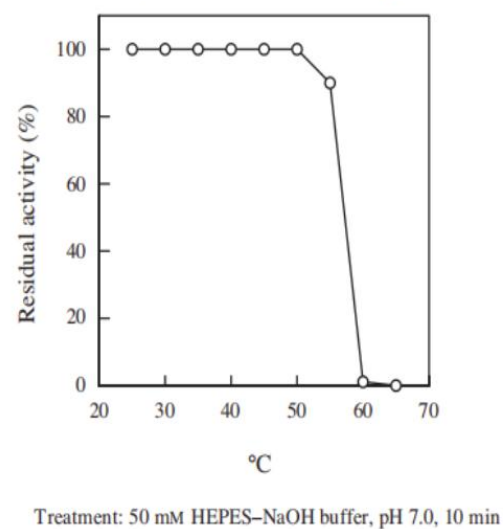
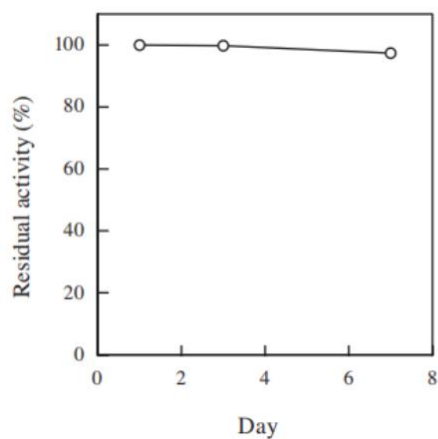


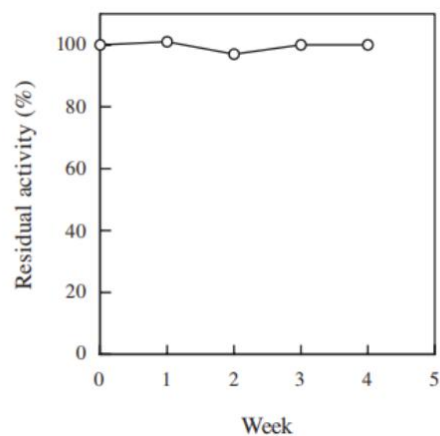


Fig. 5 Stability (liquid form) at 37°C



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

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



(Kept under dry conditions)



源叶