NIPRO ENZYMES

# PHOSPHOTRANSACETYLASE (PTA)

[EC 2. 3. 1. 8]

from *Bacillus stearothermophilus*

Acetyl-CoA + Pi ↔ AcetyIphosphate + CoA

**SPECIFICATION**

| | | | |
|---|---|---|---|
| State | : | Lyophilized | |
| Specific activity | : | more than 5,000 U/mg protein | |
| Contaminants | : | (as PTA activity = 100 %) | |
| | | Acetate kinase | < 0.01 % |
| | | Adenylate kinase | < 0.01 % |
| | | Lactate dehydrogenase | < 0.01 % |

**PROPERTIES**

| | | | |
|---|---|---|---|
| Molecular weight | : | ca. 70,000 | |
| Subunit molecular weight | : | ca. 35,000 | |
| Optimum pH | : | 7.5 | (Fig. 1) |
| pH stability | : | 7.0 - 11.0 | (Fig. 2) |
| Isoelectric point | : | 4.5 | |
| Thermal stability | : | No detectable decrease in activity up to 50 °C. | (Fig. 3, 4) |
| Michaelis constants | : | (87 mM Tris-HCl buffer, pH 7.5, at 30 °C) | |
| | | Coenzyme A | 0.4 mM |
| | | Acetyl Phosphate | 1.1 mM |

**STORAGE**

Stable at -20 °C for at least one year

**APPLICATION**

The enzyme is useful for determination of CoA or acetate.

## ASSAY

### Principle

The change in absorbance is measured at 233 nm according to the following reaction.

$$\text{Acetylphosphate + CoA} \xrightarrow{\text{PTA}} \text{Acetyl-CoA + Pi}$$

### Unit Definition

One unit of activity is defined as the amount of PTA that forms 1 μmol of acetyl-CoA per minute at 30 °C.

### Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 7.5
Ⅱ CoA solution ; 6.4 mM (50 mg CoA trilithium salt/10 mL distilled water)
Ⅲ Acetylphosphate solution ; 217 mM (0.400 g acetylphosphate potassium lithium salt/10 mL distilled water)
Ⅳ Ammonium sulfate (AmS) solution ; 1 M (13.2 g AmS/100 mL distilled water)

### Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 20 U/mL with 50 mM Tris-HCl buffer, pH 8.0.

### Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.

| | | | |
|---|---|---|---|
| Solution Ⅰ | 26.0 mL | Solution Ⅲ | 1.0 mL |
| Solution Ⅱ | 2.0 mL | Solution Ⅳ | 1.0 mL |

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 233 nm per minute ($\Delta Abs_{233}$) in the linear portion of curve.

### Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{233}) \text{ X } (3.00 + 0.01)}{4.44 \text{ X } 0.01} \text{ X d.f.}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)*}}$$

d.f. ; dilution factor
4.44 ; differential millimolar extinction coefficient between acety-CoA and CoA ($cm^2/\mu mol$)
*Protein concentration ; determined by Bradford's method

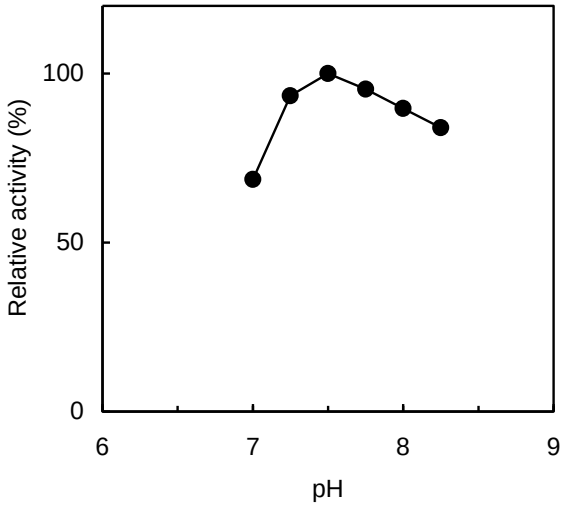


Fig. 1 pH profile

● Tris-HCl

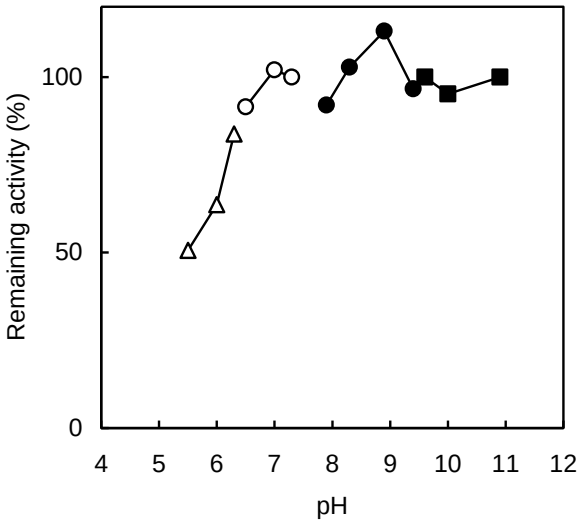


Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

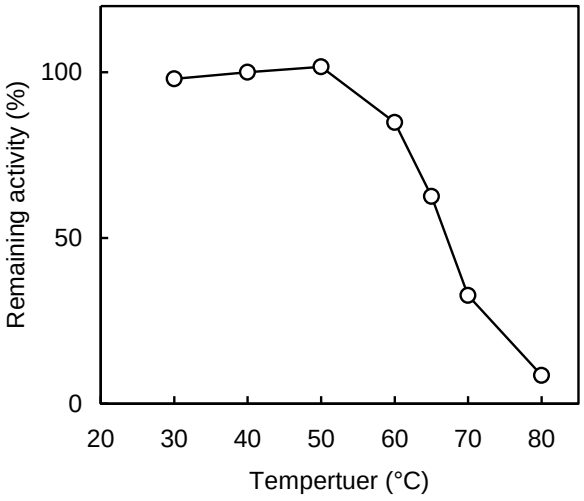


Fig. 3 Thermal stability

treated for 15 min in 50 mM Tris-HCl buffer, pH 8.0

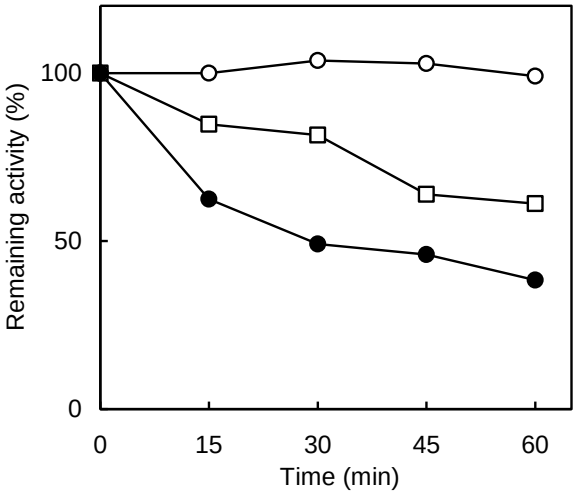


Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.0
○ 50 °C, □ 60 °C, ● 65 °C