Phosphotransacetylase

Acetyl-CoA + Pi ↔ Acetylphosphate + CoA

PTA

EC 2. 3. 1. 8

Bacillus stearothermophilus

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 : (87mM 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.

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

1. Buffer solution ; 100 mM Tris-HCl, pH 7.5
2. CoA solution ; 6.4 mM (50 mg CoA trilithium salt/10 mL distilled water)
3. Acetylphosphate solution ; 217 mM (0.400 g acetylphosphate potassium lithium salt/10 mL distilled water)
4. 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 1. 26.0 mL Solution 2. 2.0 mL Solution 3. 1.0 mL Solution 4. 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 (ΔAbs_{233}) in the linear portion of curve.

CALCULATION

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

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

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

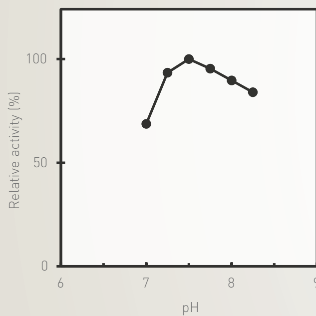

Fig. 1 pH profile

● Tris-HCl

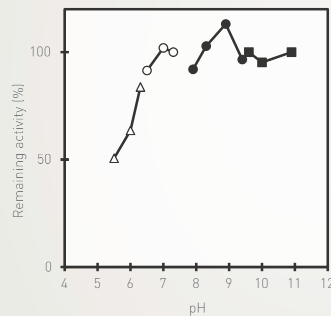

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);

△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

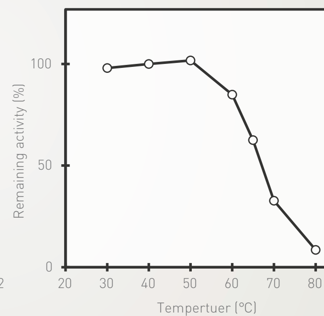

Fig. 3 Thermal stability

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

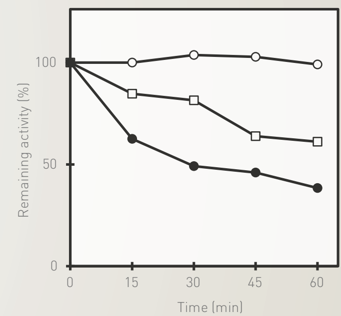

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.0

○ 50 °C, □ 60 °C, ● 65 °C