NIPRO ENZYMES

PYRUVATE KINASE (PK)

[EC 2.7.1.40]

from *Bacillus stearothermophilus*

ATP + Pyruvate ↔ ADP + Phosphoenolpyruvate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 230 U/mg protein	
Contaminants	:	(as PK activity = 100 %)	
		Adenylate kinase	< 0.01 %
		Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 260,000	
Subunit molecular weight	:	ca. 68,000	
Optimum pH	:	7.0	(Fig. 1)
pH stability	:	8.0 - 10.0	(Fig. 2)
Isoelectric point	:	5.2	
Thermal stability	:	No detectable decrease in activity up to 55 °C.	(Fig. 3, 4)
Michaelis constants	:	(76 mM Imidazole-HCl buffer, pH 7.2, at 30 °C)	
		Phosphoenolpyruvate	0.6 mM
		ADP	0.9 mM

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, ADP determination.

ASSAY

Principle

The change in absorbance is measured at 340 nm according to the following reaction.

$$\text{ADP + PEP} \xrightarrow{\text{PK}} \text{ATP + Pyruvate}$$

$$\text{Pyruvate + NADH + H}^{+} \xrightarrow{\text{LDH}} \text{Lactate + NAD}^{+}$$

Unit Definition

One unit of activity is defined as the amount of PK that forms 1 μmol of pyruvate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Imidazole-HCl, pH 7.2
Ⅱ ADP solution ; 100 mM (0.507 g ADP disodium salt·$2H_2O$/(9.0 mL distilled water + 1.0 mL 1 N NaOH))
Ⅲ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)
Ⅳ Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
Ⅴ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅵ KCl solution ; 2.5 M (18.64 g KCl/100 mL distilled water)
Ⅶ Lactate dehydrogenase (LDH) ; (from pig heart, Oriental Yeast Co. Ltd., Product Code: LDH-02) ammonium sulfate suspension, approx. 5,000 U/mL at 25 °C

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM Tris-HCl buffer, pH 8.5.

Procedure

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

Solution Ⅰ	22.71 mL	Solution Ⅴ	0.48 mL
Solution Ⅱ	2.40 mL	Solution Ⅵ	0.90 mL
Solution Ⅲ	0.45 mL	Solution Ⅶ	0.06 mL
Solution Ⅳ	3.00 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 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \times (3.00 + 0.01)}{6.22 \times 0.01} \times \text{d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH (cm^2/μmol)
*Protein concentration ; determined by Bradford's method

REFERENCE

1. Sakai, H., Suzuki, K., and Imahori, K.; *J. Biochem.*, **99**, 1157 (1986)

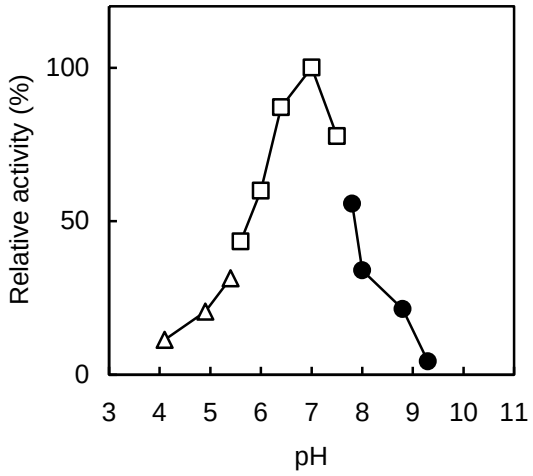

Fig. 1 pH profile

△ acetate, □ imidazole-HCl,
● Tris-HCl

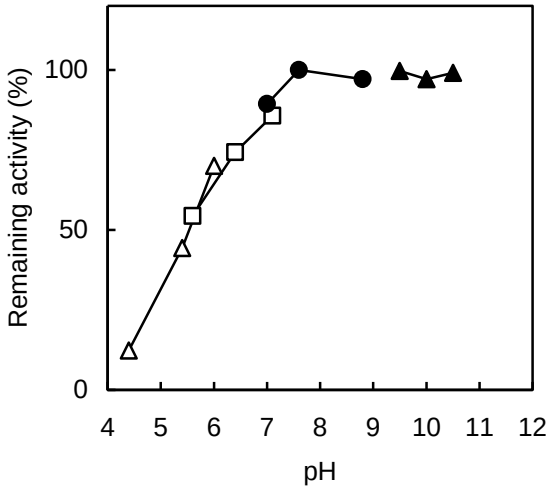

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, □ imidazole-HCl,
● Tris-HCl, ▲ carbonate

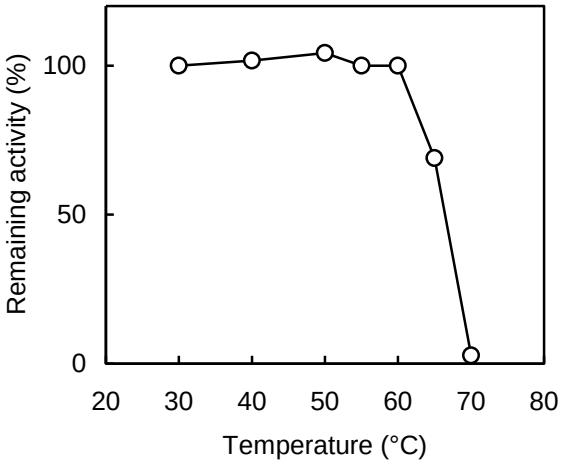

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tris-HCl buffer, pH 8.5

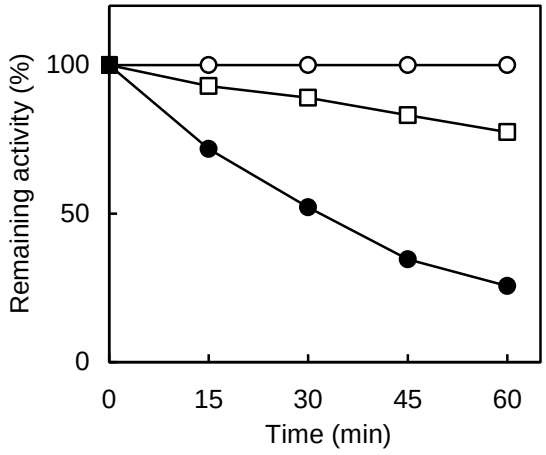

Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 8.5
○ 55 °C, □ 60 °C, ● 65 °C