Thermostable Enzymes for Clinical Chemistry

NIPRO Enzymes

NIPRO

From *Zymomonas mobilis*

ALCOHOL DEHYDROGENASE (ZM-ADH)
GLUCOKINASE (ZM-GlcK)
GLUCOSE-6-PHOSPHATE DEHYDROGENASE (ZM-G6PDH)

From *Bacillus stearothermophilus*

ACETATE KINASE (AK)
ADENYLATE KINASE (AdK)
ALANINE DEHYDROGENASE (AlaDH)
ALANINE RACEMASE (AlaR)
DIAPHORASE Ⅰ [EC 1.6.99.-] (Di-1)
GLUCOKINASE (GlcK)
α-GLUCOSIDASE (α-Glu)
LEUCINE DEHYDROGENASE (LeuDH)
PHOSPHOFRUCTOKINASE (PFK)
PHOSPHOGLUCOSE ISOMERASE (PGI)
PHOSPHOTRANSACETYLASE (PTA)
POLYNUCLEOTIDE PHOSPHORYLASE (PNPase)
PYRUVATE KINASE (PK)
SUPEROXIDE DISMUTASE (SOD)

From Others

BILIRUBIN OXIDASE (BOD3)
DIAPHORASE3 (DI-3)
DIAPHORASE22 (Di-22)
GALACTOSE DEHYDROGENASE (GalDH)
GLUCOKINASE2 (GlcK2)
GLUCOSE DEHYDROGENASE (GlcDH2)
D-LACTATE DEHYDROGENASE (D-LDH)
MALATE DEHYDROGENASE (MDH)
MUTAROTASE (MRO)
PHENYLALANINE DEHYDROGENASE (PheDH)
6-PHOSPHOGLUCONATE DEHYDROGENASE (6PGDH)
SORBITOL DEHYDROGENASE (SorDH)

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Bacillus stearothermophilus is used as a synonym of *Geobacillus stearothermophilus* .

2018.03

Quality

The Quality Management System of Enzyme Center, NIPRO Corp. has been certified as to meet the requirements of ISO9001 in the scope of design, development and manufacture of enzymes for analytical reagents and industrial use by JAPAN CHEMICAL QUALITY ASSURANCE LTD.

NIPRO ENZYMES

ALCOHOL DEHYDROGENASE (ZM-ADH)

[EC 1 .1 .1 .1]

from *Zymomonas mobilis*

Alcohol + NAD^+ ↔ Aldehyde + NADH + H^+

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 400 U/mg protein	
Contaminants	:	(as ZM-ADH activity = 100 %)	
		Glucose-6-phosphate dehydrogenase	< 0.10 %
		Glucokinase	< 0.02 %
		Pyruvate kinase	< 0.02 %
		NADH oxidase	< 0.01 %
		Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 148,000	
Subunit molecular weight	:	ca. 37,000	
Optimum pH	:	9.5 - 10.0	(Fig. 1)
pH stability	:	7.0 - 9.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 40 °C.	(Fig. 3, 4)
Michaelis constants	:	(100 mM Glycine-KOH buffer, pH 9.0, at 30 °C)	
		Ethanol	110 mM
		Methanol	350 mM
		NAD^+	0.12 mM
		Acetaldehyde	1.66 mM
		NADH	0.03 mM
Substrate specificity	:	Ethanol	100 %
		Methanol	0.05 %
		n - Propanol	42.3 %
		n - Butanol	0.28 %

STORAGE

Stable at -20 °C for at least six months

APPLICATION

The enzyme is useful for determination of alcohols or aldehydes.

ASSAY

Principle

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

$$\text{Ethanol} + NAD^+ \xrightarrow{\text{ZM-ADH}} \text{Acetaldehyde} + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of ZM-ADH that forms 1 μmol of NADH per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 80 mM Glycine-KOH, pH 9.5
Ⅱ NAD^+ solution ; 10 mM (0.0663 g NAD^+ free acid/10 mL distilled water)
Ⅲ Ethanol solution ; Ethanol (96 %)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM Tris succinate buffer containing 1mg/mL BSA and 0.2 mM $CoCl_2$, pH 7.0

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 22.90mL
   Solution Ⅱ 6.00mL
   Solution Ⅲ 1.10mL
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

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

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

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

REFERENCE

1. Neale, A.D., Scopes. R.K., Kelly, J.M., and Wettenhall, R.E.H.; *Eur. J. Biochem*., **154**, 119 (1986)

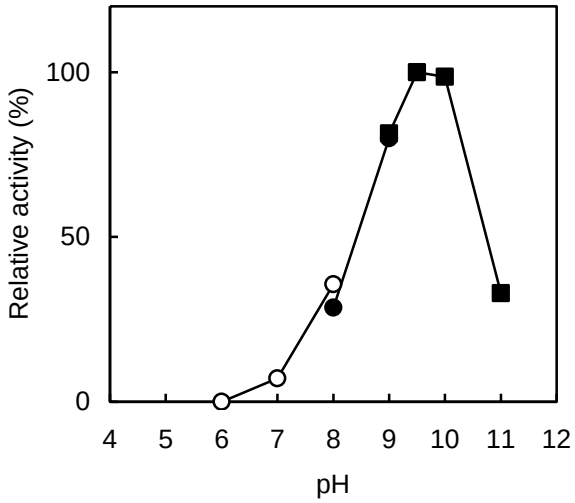

Fig. 1 pH profile

○ phosphate, ● Tris-HCl,
■ Gly-KOH

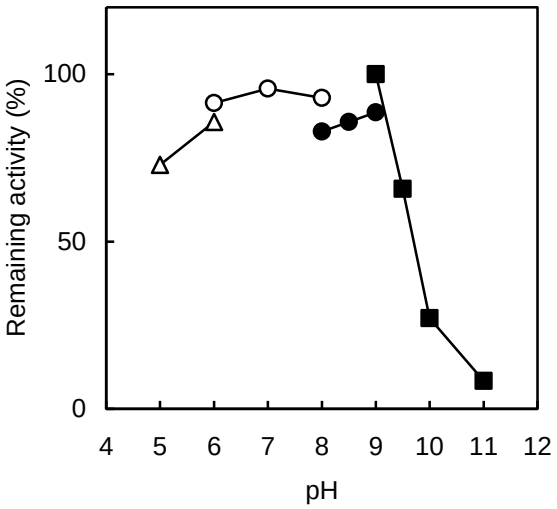

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M), containing 0.5 mM $CoCl_2$;
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

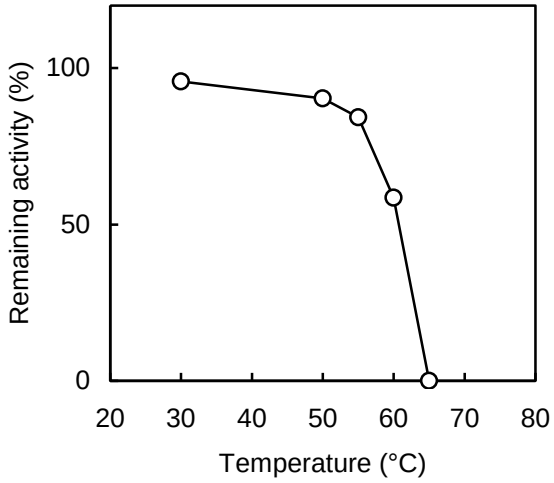

Fig. 3 Thermal stability

treated for 15 min in 0.1M phosphate buffer containing 0.2 mM $CoCl_2$, pH 6.5

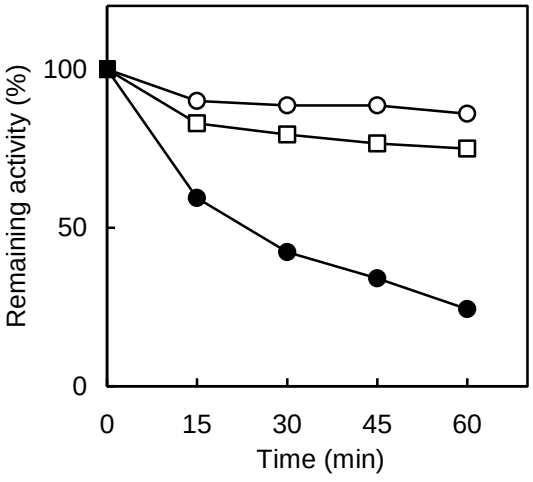

Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer containing 0.2 mM $CoCl_2$, pH 6.5
○ 50 °C, □ 55 °C, ● 60 °C

NIPRO ENZYMES

GLUCOKINASE (ZM-GlcK)

[EC 2. 7. 1. 2]

from *Zymomonas mobilis*

ATP + D-Glucose ↔ ADP + D-Glucose 6-phosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 150 U/mg protein	
Contaminants	:	(as ZM-GlcK activity = 100 %)	
		Glucose-6-phosphate dehydrogenase	< 0.02 %
		Phosphoglucomutase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 66,000	
Subunit molecular weight	:	ca. 33,000	
Optimum pH	:	7.0 - 8.0	(Fig. 1)
pH stability	:	6.0 - 8.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 40 °C.	(Fig. 3, 4)
Michaelis constants	:	(60mM Phosphate buffer, pH 7.0, at 30 °C)	
		Glucose	0.10 mM
		ATP	0.65 mM
Activator	:	Pi	

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.
Tris-HCI buffer is not suitable for the practical use of ZM-GlcK.

ASSAY

Principle

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

$$\text{ATP + Glucose} \xrightarrow{\text{ZM-GlcK}} \text{ADP + Glucose 6-phosphate}$$

$$\text{Glucose 6-phosphate + NAD}^+ \xrightarrow{\text{G6PDH}} \text{Gluconolactone 6-phosphate + NADH + H}^+$$

Unit Definition

One unit of activity is defined as the amount of ZM-GlcK that forms 1 μmol of glucose 6-phosphate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Triethanolamine - NaOH and 3 mM K_2HPO_4, pH 7.5
Ⅱ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅲ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅳ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)
Ⅴ Glucose solution ; 40mM (0.072 g glucose (anhyd.)/10 mL distilled water)
Ⅵ Glucose-6-phosphate dehydrogenase (G6PDH) ; 2000 U/mL (from *Zymomonas mobilis*, Nipro Corp., Dissolve with Buffer solution Ⅰ)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM potassium phosphate buffer containing 1 mg/mL BSA, pH 7.0.

Procedure

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

Solution Ⅰ	20.07mL	SolutionⅣ	0.60mL
Solution Ⅱ	1.50mL	Solution Ⅴ	7.50mL
SolutionⅢ	0.30mL	SolutionⅥ	0.03mL

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

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

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

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

REFERENCE

1. Scopes. R.K., Testolin, V., Stoter, A., Griffiths-Smith, K., and Algar, E.M.; *Biochem. J.*, **228**, 627 (1985)

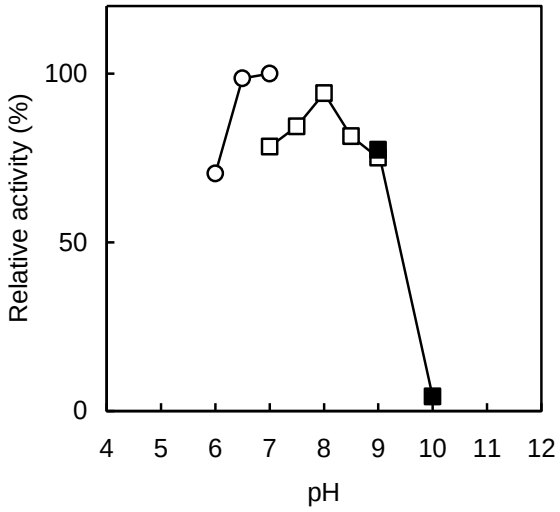

Fig. 1 pH profile

○ MES-KOH, □ TEA-NaOH,
■ Gly-KOH

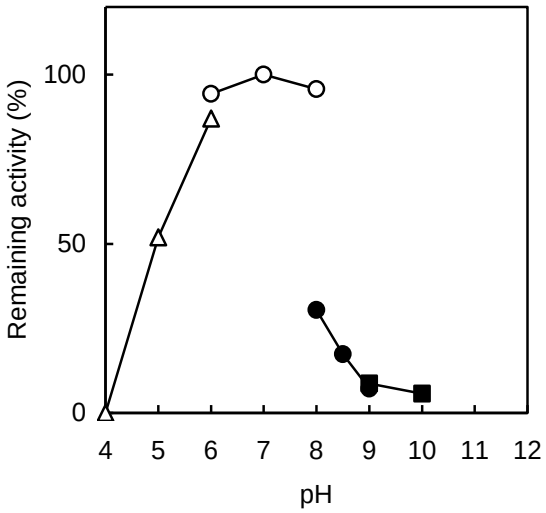

Fig. 2 pH stability

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

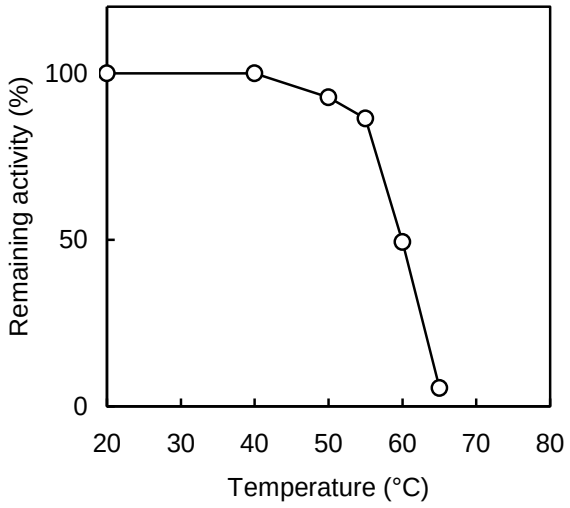

Fig. 3 Thermal stability

treated for 15 min in 0.1 M phosphate buffer, pH 7.0

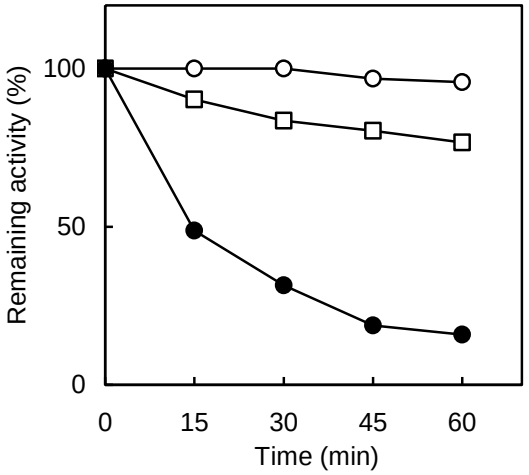

Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer , pH 7.0
○ 40 °C, □ 50 °C, ● 60 °C

NIPRO ENZYMES

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (ZM-G6PDH)

[EC 1. 1. 1. 49]

from *Zymomonas mobilis*

D-Glucose 6-phosphate + $NAD(P)^+$ ↔ D-Gluconolactone 6-phosphate + $NAD(P)H + H^+$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 250 U/mg protein	
Contaminants	:	(as ZM-G6PDH activity = 100 %)	
		Glucokinase	< 0.02 %
		Phosphoglucomutase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.02 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 208,000	
Subunit molecular weight	:	ca. 52,000	
Optimum pH	:	8.0	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	:	(30mM Tris-HCl buffer, pH 8.0, at 30 °C)	
		Glucose 6-phosphate	0.14 mM
		$NADP^+$	0.02 mM
		NAD^+	0.14 mM
Substrate specificity	:	$NADP^+$	70 %
		NAD^+	100 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.

ASSAY

Principle

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

$$\text{Glucose 6-phosphate} + NAD^+ \xrightarrow{\text{ZM-G6PDH}} \text{Gluconolactone 6-phosphate} + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of ZM-G6PDH that forms 1 μmol of NADH per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 50 mM Tris-HCl, pH 8.0
Ⅱ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)
Ⅲ Glucose 6-phosphate (G6P) solution ; 33 mM (0.112 g G6P disodium salt $2H_2O$/10mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50mM potassium phosphate buffer containing 1 mg/mL BSA, pH 7.0.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 26.40mL
   Solution Ⅱ 0.90mL
   Solution Ⅲ 2.70mL
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 340nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

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

REFERENCE

1. Scopes, R.K., Testolin, V., Stoter, A., Griffiths-Smith, K., and Algar. E.M.; *Biochem. J.*, **228**. 627 (1985)

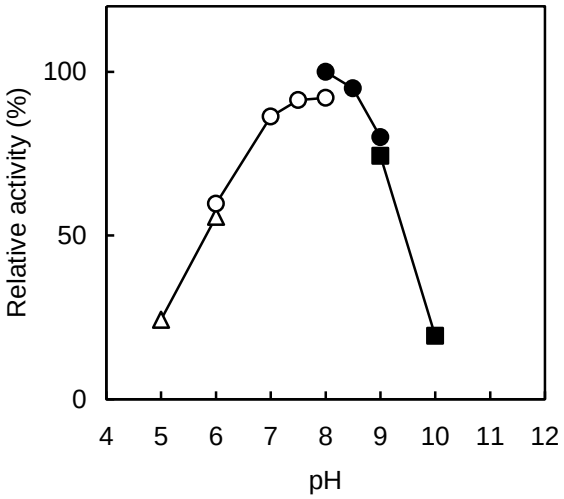

Fig. 1 pH profile

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

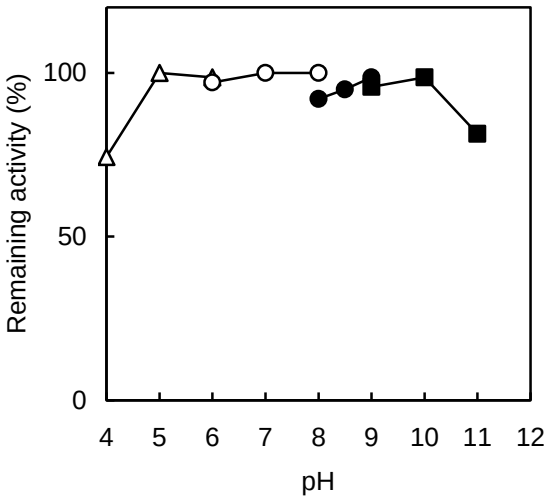

Fig. 2 pH stability

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

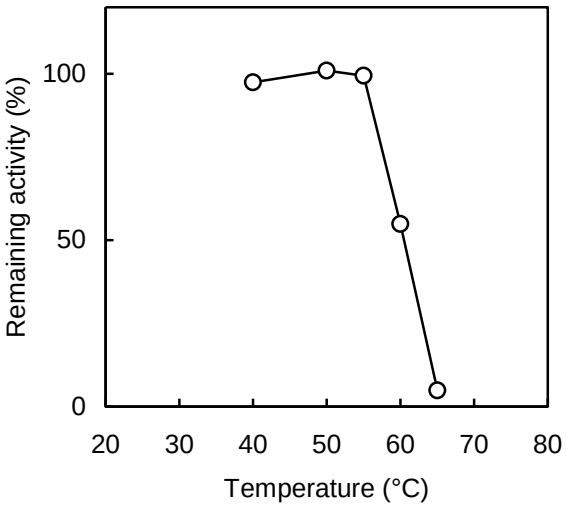

Fig. 3 Thermal stability

treated for 15 min in 0.1 M phosphate buffer, pH 7.0

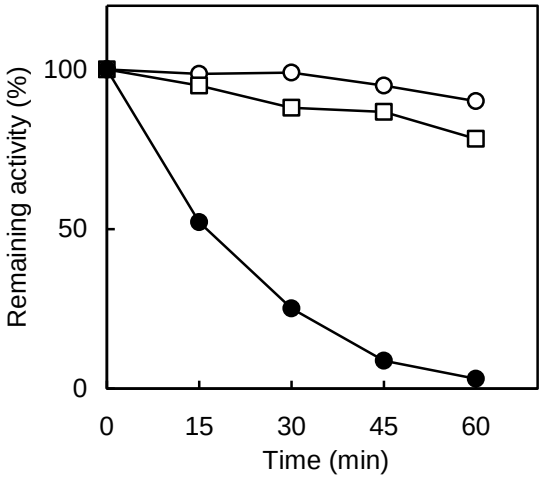

Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer, pH 7.0
○ 50 °C, □ 55 °C, ● 60 °C

NIPRO ENZYMES

ACETATE KINASE (AK)

[EC 2. 7. 2. 1]

from *Bacillus stearothermophilus*

ATP + Acetate ↔ ADP + AcetyIphosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 1,100 U/mg protein	
Contaminants	:	(as AK activity = 100 %)	
		Lactate dehydrogenase	< 0.01 %
		Adenylate kinase	< 0.01 %
		NADH oxidase	< 0.01 %
		GOT	< 0.01 %
		GPT	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 160,000	
Subunit molecular weight	:	ca. 40,000	
Optimum pH	:	7.2	(Fig. 1)
pH stability	:	7.0 - 8.0	(Fig. 2)
Isoelectric point	:	4.8	
Thermal stability	:	No detectable decrease in activity up to 65 °C.	(Fig. 3, 4)
Michaelis constants	:	(57 mM Imidazole- HCI buffer, pH 7.2, at 30 °C)	
		Acetate	120 mM
		AcetyIphosphate	2.3 mM
		ATP	1.2 mM
		ADP	0.8 mM
Substrate specificity	:	Acetate	100 %
		Formate	0 %
		Propionate	5 %
		Butyrate	0 %
		Oxalate	0 %
		Citrate	0 %
		Malate	0 %
		Glycine	0 %
Activator	:	Fructose 1,6-bisphosphate	

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of acetate or for ATP regeneration system.

ASSAY

Principle

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

$$\text{ATP + Acetate} \xrightarrow{\text{AK}} \text{ADP + Acetylphosphate}$$

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

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

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 100 mM Imidazole-HCl, pH 7.2
Ⅱ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅲ Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
Ⅳ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/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 Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10mg/mL) approx. 200 U/mg at 25 °C
Ⅷ 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
Ⅸ Sodium acetate solution ; 2 M (27.22g sodium acetate·$3H_2O$/100 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.4 mL of reaction mixture into a cuvette.

Solution Ⅰ	16.92mL	Solution Ⅴ	0.60mL
Solution Ⅱ	3.00mL	Solution Ⅵ	0.90mL
Solution Ⅲ	1.80mL	Solution Ⅶ	0.12mL
Solution Ⅳ	0.60mL	Solution Ⅷ	0.06mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.60 mL of Solution Ⅸ and 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

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

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

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

REFERENCE

1. Nakajima, H., Suzuki, K., and Imahori, K. ; *J. Biochem*., **84**, 193 (1978)
2. Nakajima, H., Suzuki, K., and Imahori, K. ; *ibid*., **84**, 1139 (1978)
3. Nakajima, H., Suzuki, K., and Imahori, K. ; *ibid*., **86**, 1169 (1979)

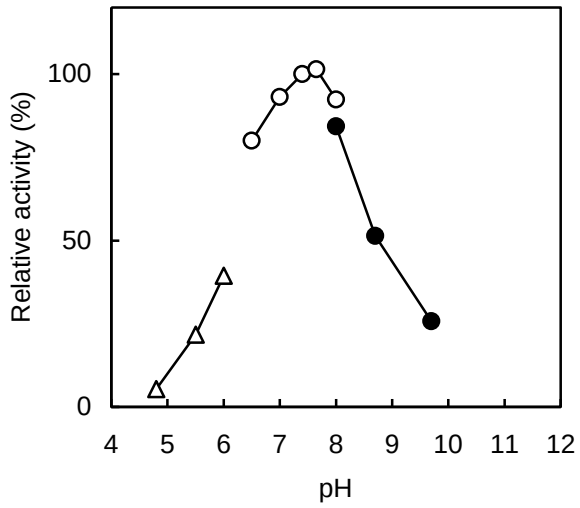

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl

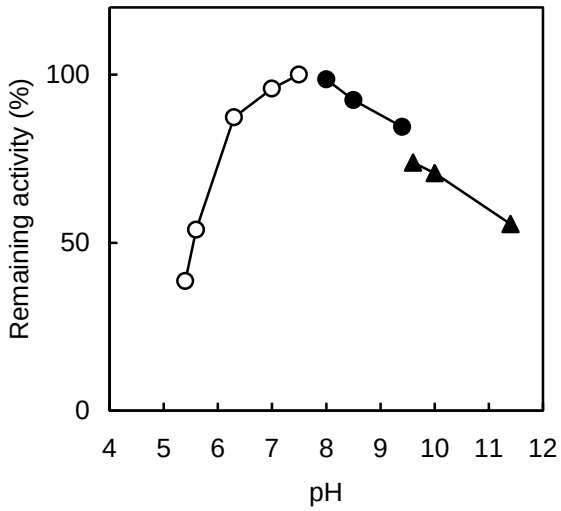

Fig. 2 pH stability

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

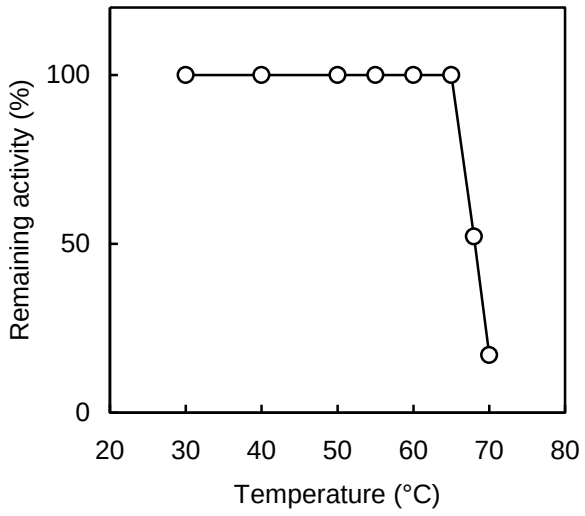

Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

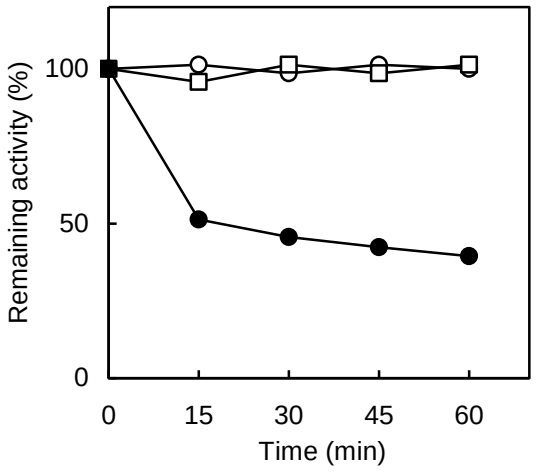

Fig. 4 Thermal stability

treated in 0.1M potassium phosphate buffer, pH 7.5
○ 60 °C, □ 65 °C, ● 70 °C

NIPRO ENZYMES

ADENYLATE KINASE (AdK)

[EC 2. 7. 4. 3]

from *Bacillus stearothermophilus*

ATP + AMP ↔ 2 ADP

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 200 U/mg protein	
Contaminants	:	(as AdK activity = 100 %)	
		ATPase	< 0.01 %
		Phosphoglycerate kinase	< 0.10 %

PROPERTIES

Molecular weight	:	ca. 20,000	
Optimum pH	:	6.5	(Fig. 1)
pH stability	:	8.0 - 10.5	(Fig. 2)
Isoelectric point	:	5.0	
Thermal stability	:	No detectable decrease in activity up to 65 °C.	(Fig. 3, 4)
Michaelis constants	:	(89 mM Imidazole-HCI buffer, pH 6.5, at 30 °C)	
		ATP	0.04 mM
		ADP	0.05 mM
		AMP	0.02 mM

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of AMP or for system involving ATP regeneration.

ASSAY

Principle

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

$$ATP + AMP \xrightarrow{AdK} 2\ ADP$$

$$2\ ADP + 2PEP \xrightarrow{PK} 2\ ATP + 2\ Pyruvate$$

$$2\ Pyruvate + 2\ NADH + 2\ H^+ \xrightarrow{LDH} 2\ Lactate + 2\ NAD^+$$

Unit Definition

One unit of activity is defined as the amount of AdK that forms 2 μmol of ADP per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Imidazole-HCl, pH 6.5
Ⅱ AMP solution ; 50 mM (0.250 g AMP disodium salt·$6H_2O$/10 mL distilled water)
Ⅲ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅳ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$ /10 mL distilled water)
Ⅴ Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
Ⅵ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅶ KCl solution ; 2.5 M (18.64 g KCl/100mL distilled water)
Ⅷ Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C
Ⅸ 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 2.5 to 5 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	26.70mL	Solution Ⅵ	0.60mL
Solution Ⅱ	0.24mL	Solution Ⅶ	1.20mL
Solution Ⅲ	0.30mL	Solution Ⅷ	0.09mL
Solution Ⅳ	0.60mL	Solution Ⅸ	0.09mL
Solution Ⅴ	0.18mL		

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

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \times (3.00 + 0.01)}{2 \times 6.22 \times 0.01} \times \text{d.f.}$$

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

d.f. ; dilution factor
2 ; according to the reaction that forms 2 μmol of ADP, one unit of activity of Adk is defined to form 2 μmol of ADP.
6.22 ; millimolar extinction coefficient of NADH (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Imahori, K., Nakajima, H., Nagata, K., and Iwasaki, T.; *Seikagaku*, **53**, 829 (1981)

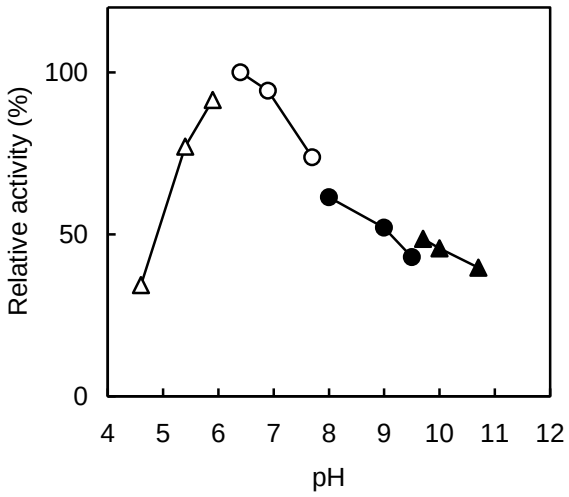

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ▲ carbonate

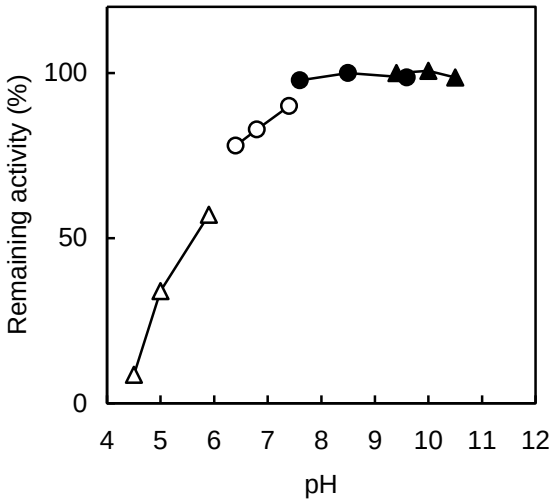

Fig. 2 pH stability

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

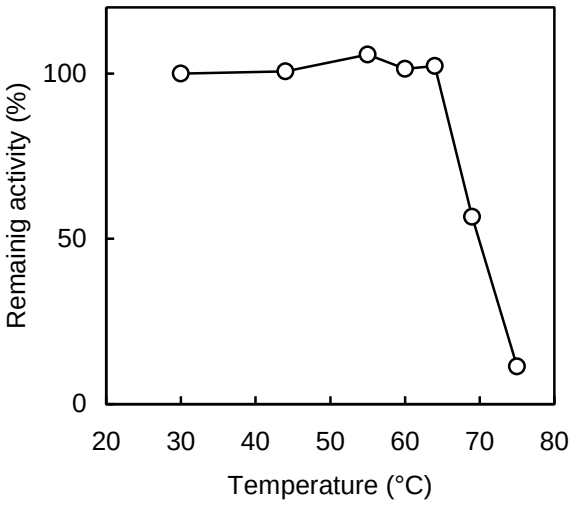

Fig. 3 Thermal stability

treated for 15 min in 0.1M Tris-HCl buffer, pH 9.0

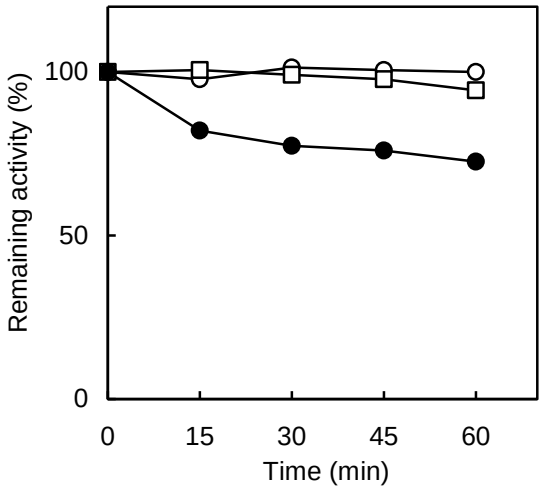

Fig. 4 Thermal stability

treated in 0.1M Tris-HCl buffer, pH 9.0
○ 60 °C, □ 65 °C, ● 70 °C

NIPRO ENZYMES

ALANINE DEHYDROGENASE (AlaDH)

[EC 1. 4. 1. 1]

from *Bacillus stearothermophilus*

$$\text{L-Alanine} + NAD^+ + H_2O \leftrightarrow \text{Pyruvate} + NH_4^+ + NADH$$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 55 U/mg protein	
Contaminants	:	(as AlaDH activity = 100 %)	
		NADH oxidase	< 0.01 %
		Lactate dehydrogenase	< 0.10 %

PROPERTIES

Molecular weight	:	ca. 230,000	
Subunit molecular weight	:	ca. 38,000	
Optimum pH	:	10.4	(Fig. 1)
pH stability	:	7.0 - 11.5	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 70 °C.	(Fig. 3, 4)
Michaelis constants	:	(125 mM Glycine-NaOH buffer, pH 10.5, at 30 °C)	
		L-Alanine	10.0 mM
		NAD^+	0.26 mM
Substrate specificity	:	L-Alanine	100 %
		L-Leucine	0 %
		L-Isoleucine	0 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of L-alanine.

ASSAY

Principle

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

$$\text{L-Alanine} + NAD^+ + H_2O \xrightarrow{\text{AlaDH}} \text{Pyruvate} + NH_4^+ + NADH$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 250 mM Glycine-NaOH, pH 10.5

Ⅱ L-Alanine solution ; 150 mM (1.336 g L-alanine/80 mL distilled water, adjusted to pH 10.5 with 1 N-NaOH and filled up to 100 mL with distilled water)

Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+/ 10 mL with distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 100 mM glycine - NaOH buffer, pH 9.5.

Procedure

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

Solution Ⅰ	15.00mL	Solution Ⅲ	1.50mL
Solution Ⅱ	10.00mL	H_2O	3.50mL

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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Sakamoto, Y., Nagata, S., Esakl, N., Tanaka, H. and Soda, K.; *J. Ferment. Bioeng.*, **69**, 154 (1990)

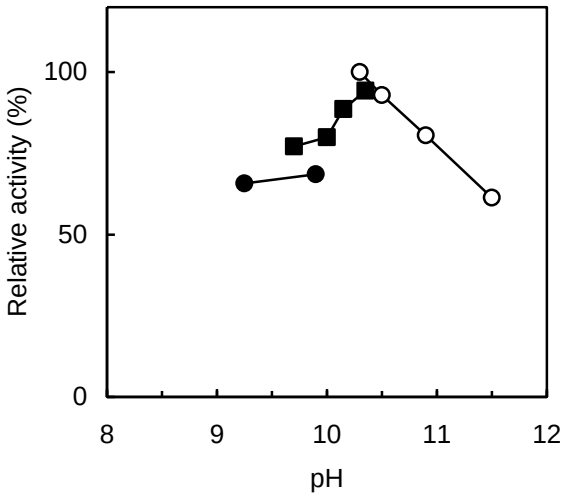

Fig. 1 pH profile

● Tris-HCl, ■ Gly-KOH,
○ phosphate

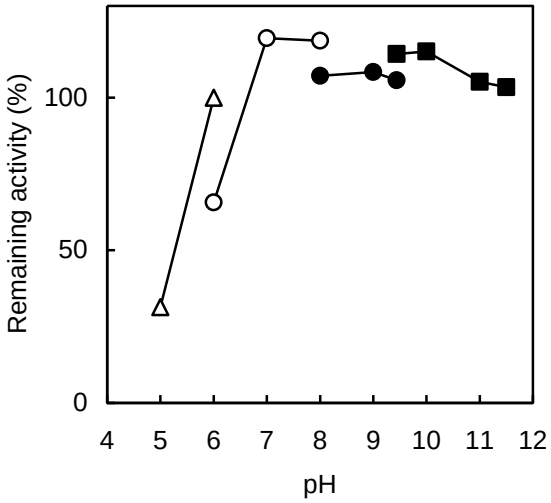

Fig. 2 pH stability

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

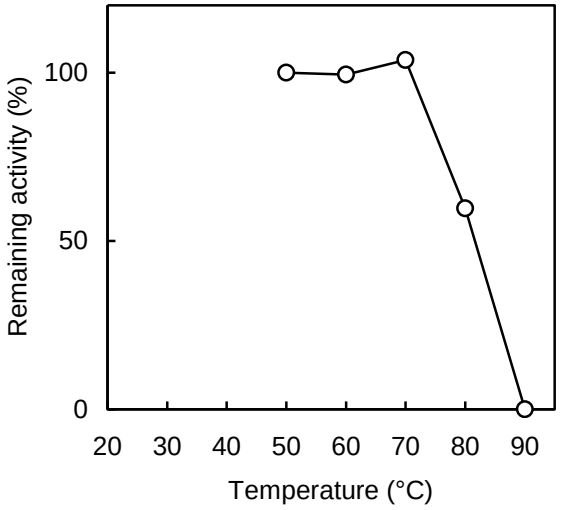

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Gly-KOH buffer, pH 9.0

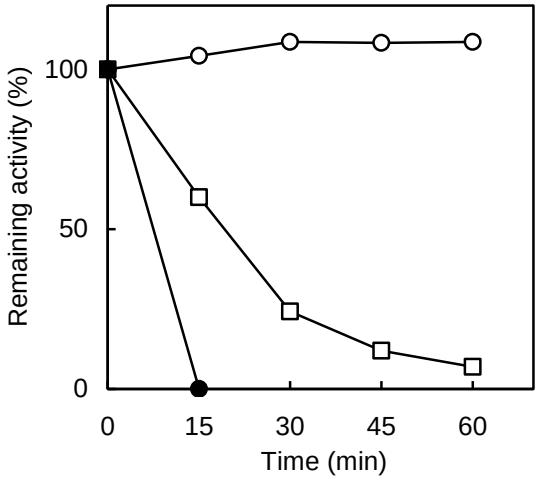

Fig. 4 Thermal stability

treated in 0.1 M Gly-KOH buffer, pH 9.0
○ 70 °C, □ 80 °C, ● 90 °C

NIPRO ENZYMES

ALANINE RACEMASE (AlaR)

[EC 5. 1. 1. 1]

from *Bacillus stearothermophilus*

D-Alanine ↔ L-Alanine

SPECIFICATION

State	:	Liquid	
Specific activity	:	more than 950 U/mg protein	
Contaminants	:	(as AlaR activity = 100 %)	
		Lactate dehydrogenase	< 0.01 %
		NADH oxidase	< 0.01 %
		Alanine dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 78,000	
Subunit molecular weight	:	ca. 39,000	
Optimum pH	:	10.5 - 12.0	(Fig. 1)
pH stability	:	5.5 - 11.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 70 °C.	(Fig. 3, 4)
Michaelis constants	:	(100 mM Carbonate buffer, pH 10.5, at 30 °C)	
		D-Alanine	31 mM
Substrate specificity	:		

STORAGE

Stable at least one year at -25 °C.

ASSAY

Principle

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

$$\text{D-Alanine} \xrightarrow{\text{AlaR}} \text{L-Alanine}$$

$$\text{L-Alanine} + NAD^+ + H_2O \xrightarrow{\text{AlaDH}} \text{Pyruvate} + NH_4^+ + \text{NADH}$$

Unit Definition

One unit of activity is defined as the amount of AlaR that forms 1 μmol of L-alanine per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 200 mM Sodium hydrogencarbonate, pH 10.5
Ⅱ D-Alanine solution ;1 M (0.891 g D-alanine/10 mL distilled water)
Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+/10 mL distilled water)
Ⅳ L-Alanine dehydrogenase (AlaDH) ; 1000 U/mL (from *Bacillus stearothermophilus*, Nipro Corp., Dissolve with distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

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

Solution Ⅰ	16.50mL	SolutionⅣ	1.50mL
Solution Ⅱ	3.00mL	H_2O	8.25mL
SolutionⅢ	0.75mL		

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

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

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

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

REFERENCE

1. Inagaki, K., Tanizawa, K., Badet, B., Walsh, C.T., Tanaka, H., and Soda, K.; *Biochemistry*, **25**, 3268 (1986)

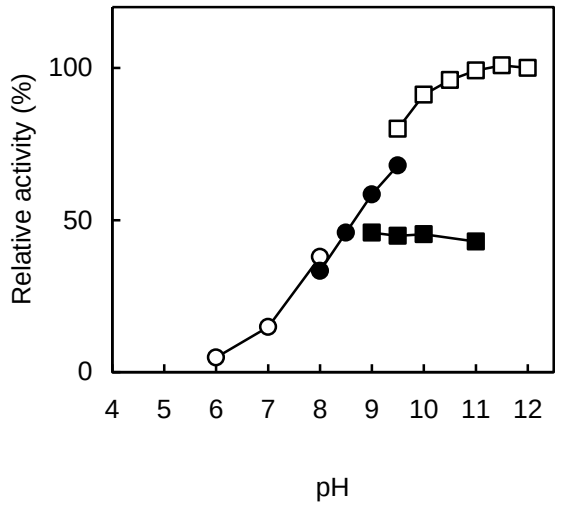

Fig. 1 pH profile

○ phosphate, ● Tris-HCl,
■ Gly-KOH, □ $NaHCO_3$-NaOH

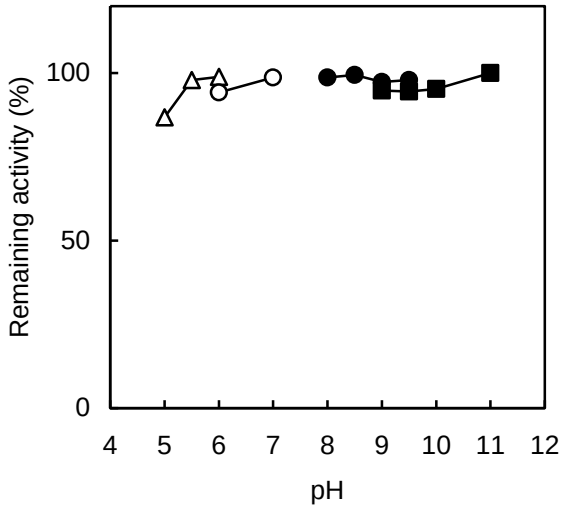

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.2 M);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

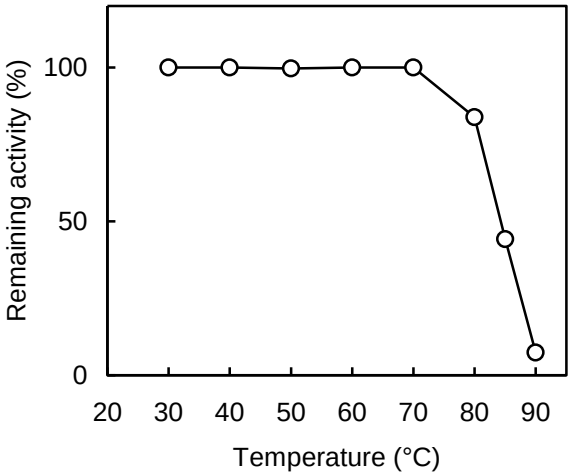

Fig. 3 Thermal stability

treated for 15 min in 50 mM Tris-HCl buffer, pH 9.0

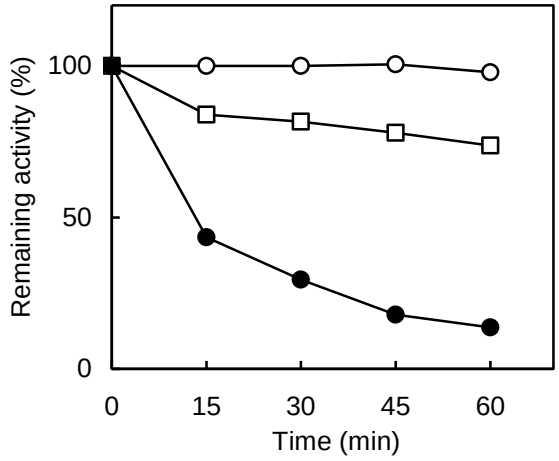

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 9.0
○ 70 °C, □ 80 °C, ● 85 °C

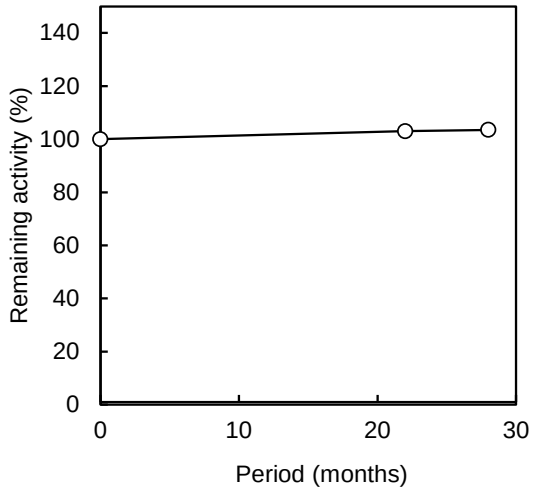

Fig. 5 Stability (Liquid form) at -25 °C

NIPRO ENZYMES

DIAPHORASE I (Di-1)

[EC 1. 6. 99. -]

from *Bacillus stearothermophilus*

$$NAD(P)H + Acceptor(ox.) + H^{+} \leftrightarrow NAD(P)^{+} + Acceptor(red.)$$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 1,000 U/mg protein	
Contaminants	:	(as Diaphorase activity = 100 %)	
		Adenylate kinase	< 0.01 %
		NADH oxidase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 30,000	
Optimum pH	:	8.0	(Fig. 1)
pH stability	:	7.5 - 9.5	(Fig. 2)
Isoelectric point	:	4.7	
Optimum temperature	:	70 °C	
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	:	See Table 1	
Substrate specificity	:	See Table 1	
Effectors	:	cations and anions	(Fig. 5, 6)

STORAGE

Stable at -20 to 5 °C for at least one year

APPLICATION

The enzyme is useful for the measurement of various dehydrogenase reactions in visible spectral range.

ASSAY

Principle

The change in absorbance is measured at 600 nm according to the following reaction.

$$NAD(P)H + DCIP(ox.) + H^+ \xrightarrow{Di\text{-}1} NAD(P)^+ + DCIP(red.)$$

Unit Definition

One unit of activity is defined as the amount of Di-1 that reduces 1 μmol of DCIP per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 500 mM Tris-HCl, pH8.5

Ⅱ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·3H_2O/10 mL distilled water)

Ⅲ 2,6-Dichlorophenolindophenol (DCIP) solution ; 1.2 mM (2.0 mg DCIP sodium salt·2H_2O/5mL distilled water) (prepare freshly)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 1.0 to 2.0 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.85 mL of reaction mixture into a cuvette.

Solution Ⅰ	3.00mL
Solution Ⅱ	2.28mL
H_2O	23.22mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.15 mL of Solution Ⅲ and 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 600 nm per minute (ΔAbs(test)) in linear portion of curve. Repeat the Procedure 3 using distilled water in place of enzyme solution, and ΔAbs(blank) is obtained.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs\ (test) - \Delta Abs\ (blank)) \times (3.00 + 0.01)}{19 \times 0.01} \times d.f.$$

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

d.f. ; dilution factor

19 ; millimolar extinction coefficient of DCIP (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Mains, I., Power, D.M., Thomas, E.W. and Buswell J. A.; *Biochem. J.*, **191**, 457 (1980)

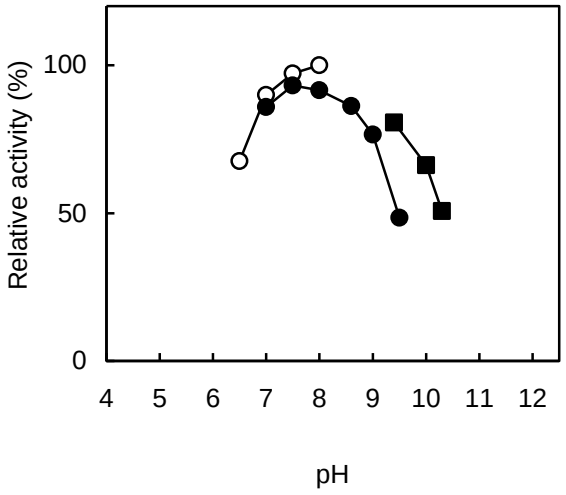

Fig. 1 pH profile

○ phosphate, ● Tris-HCl,
■ Gly-KCl-KOH

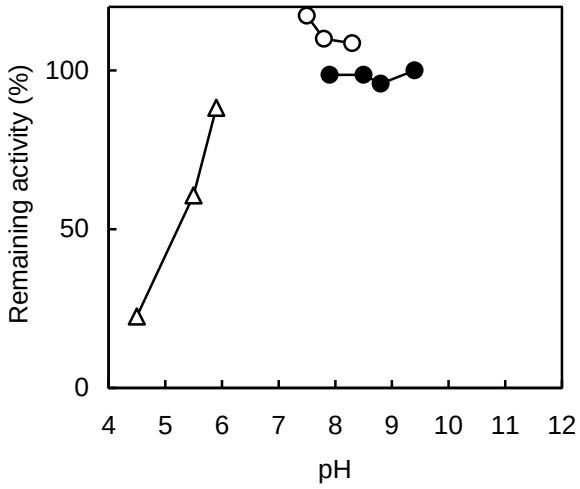

Fig. 2 pH stability

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

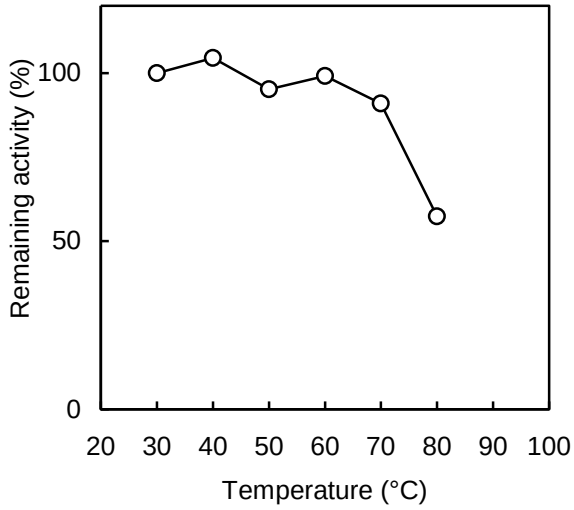

Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

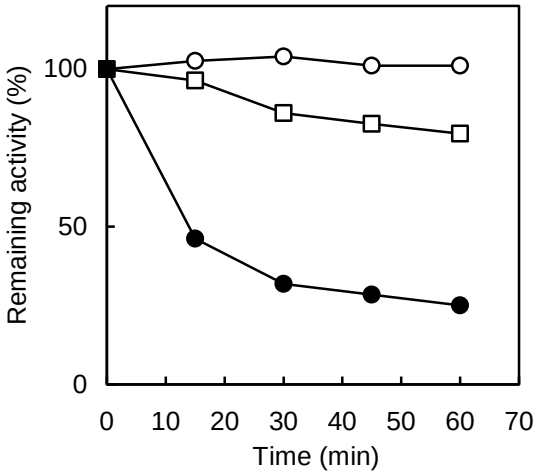

Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.5
○ 50 °C, □ 60 °C, ● 70 °C

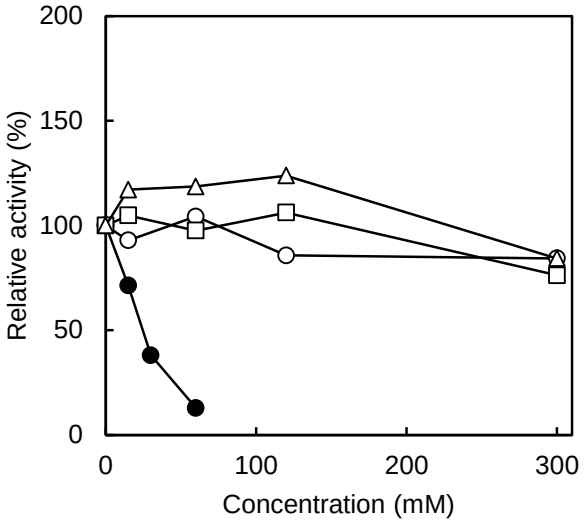

Fig. 5 Effect of various cations on the activity of DIAHORASE

Measurement : 0.30 mL of each cation solution and 3.00 mL of assay mixture were mixed, and incubated at 30° C for about 3 minutes. After incubation, 0.01mL of enzyme solution was added to the reaction mixture and the activity of DIAPHORASE was measured.
○ NaCl, △ KCl, □ $MgCl_2$, ● $CaCl_2$

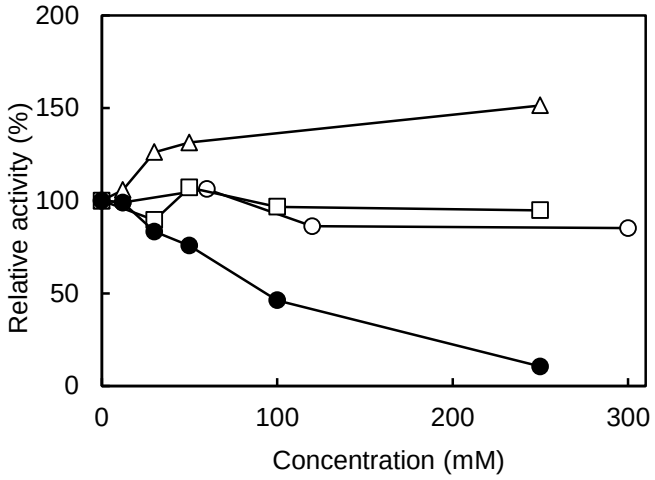

Fig. 6 Effect of various anions on the activity of

Measurement : 0.30 mL of each anion solution and 3.00 mL of assay mixture were mixed, and incubated at 30° C for about 3 minutes. After incubation, 0.01 mL of enzyme solution was added to the reaction mixture and the activity of DIAPHORASE was measured.
○ NaCl, △CH_3COONa, □ Na_2SO_4, ●$NaHCO_3$

NIPRO ENZYMES

Table 1. SUBSTRATE SPECIFICITY OF DIAPHORASE

Acceptor	DCIP[*1]	NBT[*2]	INT[*3]	FMN[*4]
$Km^{Acceptor}$ (mM)	0.015	0.15	0.40	-
Km^{NADH} (mM)	0.50	0.02	0.07	-
Km^{NADPH} (mM)	0.52	0.19	0.50	-
Optimum pH	8.0	> 10	7.5	< 6.5
Activity ^{NADH} (U/mg)	1,200	225	290	18
Activity ^{NADPH} (U/mg)	4	150	120	-
Assay Mixture	Tris-HCl (pH 8.5) 50 mM NAD(P)H 1 mM DCIP 0.06 mM	TEA (pH 7) 50 mM NAD(P)H 1 mM NBT 0.5 mM Triton X-100 0.1 %	Phosphate (pH 7.5) 96 mM NAD(P)H 1 mM INT 3 mM DMSO[*6] 2 % BSA[*5] 1 mg/mL	Phosphate (pH 7) 88 mM NADH 0.2 mM FMN 0.13 mM
Wavelength for measurement (nm)	600	550	492	340
Extinction coefficient (cm^2/μmol)	19	12.4	19.2	6.2

*1 2,6-Dichlorophenolindophenol
*2 Nitro blue tetrazolium
*3 *p*-Iodonitrotetrazolium violet
*4 Flavin mononucleotide
*5 Bovine serum albumin
*6 Added 1/40 volume of 120mM INT (0.607g/10mL 80% DMSO) into the Assay Mixture

Effect of BSA on the activity of DIAPHORASE: (See next page)

BSA stimulates the activity with INT as electron acceptor and the activation can be increased 30 fold with concentrations above 1 mg/mL BSA (Fig. 10). The extent of activation for DCIP is about 35 %, whereas the activities with NBT and FMN are not affected by BSA.

Effect of Triton X-100 on the activity of DIAPHORASE: (See next page)

The activity with NBT is little in the absence of Triton X-100, but is greatly increased by the addition of Triton X-100 (Fig. 8). On the other hand, Triton X-100 has no effect on the activities with DCIP, INT and FMN.

NBT (Nitro blue tetrazolium)

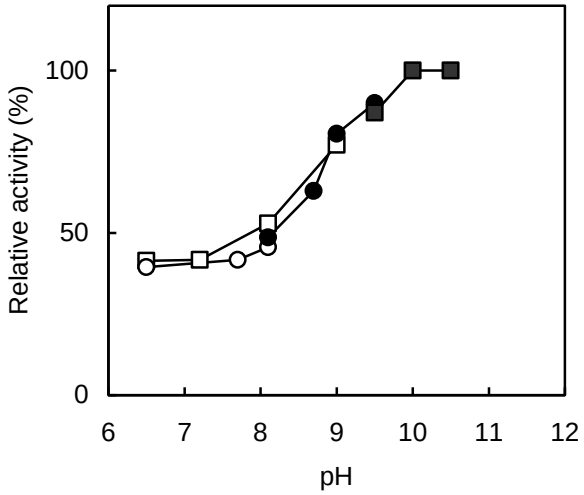

Fig. 7 pH profile

□ triethanolamine, ○ phosphate,
● Tris-HCl, ■ Gly-KCl-KOH

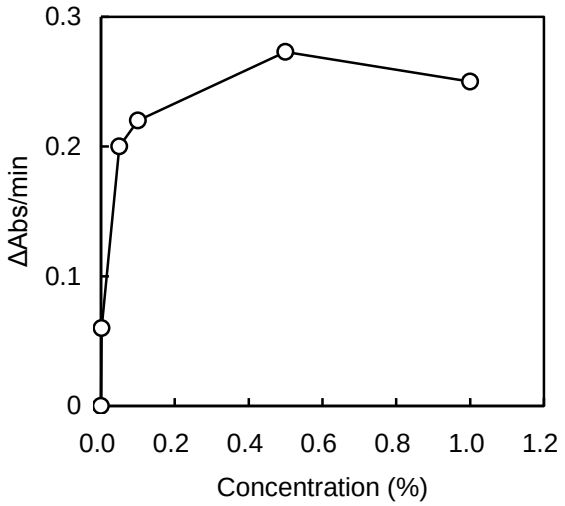

Fig. 8 Effect of Trion- X-100 on the activity of DIAPHORASE

INT (*p*-Iodonitrotetrazolium violet)

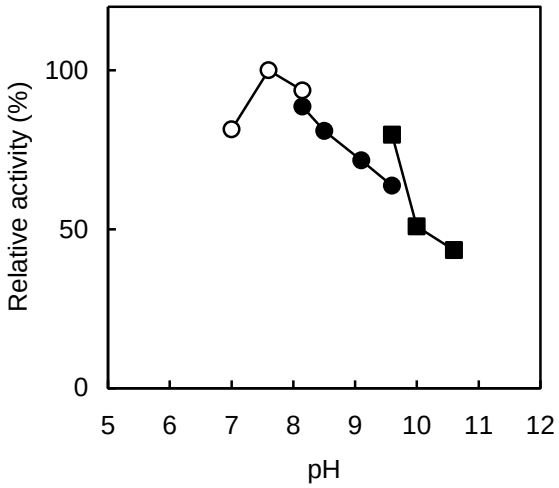

Fig. 9 pH profile

○ phosphate, ● Tris-HCl,
■ Gly-KOH,

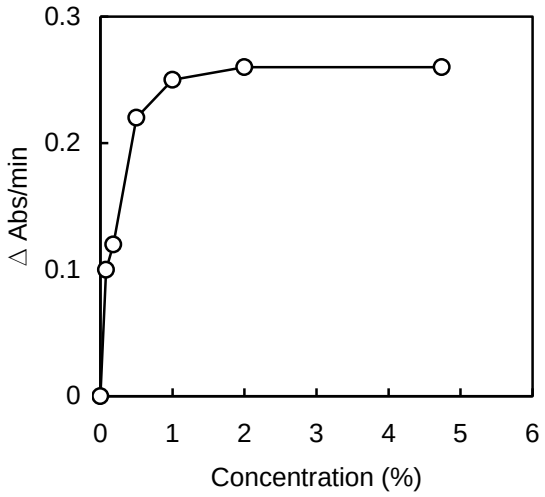

Fig. 10 Effect of BSA on the activity of DIAPHORASE

NIPRO ENZYMES

GLUCOKINASE (GlcK)

[EC 2. 7. 1. 2]

from *Bacillus stearothermophilus*

ATP + D-Glucose ↔ ADP + D-Glucose 6-phosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 350 U/mg protein	
Contaminants	:	(as GlcK activity = 100 %)	
		Glucose-6-phosphate dehydrogenase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 68,000	
Subunit molecular weight	:	ca. 32,000	
Optimum pH	:	8.5	(Fig. 1)
pH stability	:	8.0 - 11.0	(Fig. 2)
Isoelectric point	:	5	
Optimum temperature	:	65 °C	
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(60mM Tris-HCI buffer, pH 8.5, at 30 °C)	
		Glucose	0.1 mM
		ATP	0.05 mM
Substrate specificity	:	D-Glucose	100 %
		D-Mannose	25 %
		D-Fructose	0 %

STORAGE

Stable at -20 to 5 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.

ASSAY

Principle

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

$$\text{ATP + Glucose} \xrightarrow{\text{GlcK}} \text{ADP + Glucose 6-phosphate}$$

$$\text{Glucose 6-phosphate + NADP}^+ \xrightarrow{\text{G6PDH}} \text{Gluconolactone 6-phosphate + NADPH + H}^+$$

Unit Definition

One unit of activity is defined as the amount of GlcK that forms 1 μmol of glucose 6-phosphate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 9.0
Ⅱ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅲ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅳ $NADP^+$ solution ; 22.5 mM mM [(0.172 g $NADP^+$ monosodium salt or 0.177 g $NADP^+$ disodium salt)/10 mL distilled water]
Ⅴ Glucose solution ; 40 mM (0.072 g glucose (anhyd.)/10 mL distilled water)
Ⅵ Glucose-6-phosphate dehydrogenase (G6PDH) ; (from yeast. Roche Diagnostics K.K., No. 127 671) suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/2 mL) approx. 140 U/mg 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	17.97mL	SolutionⅣ	1.20mL
Solution Ⅱ	1.20 mL	Solution Ⅴ	9.00mL
SolutionⅢ	0.60 mL	SolutionⅥ	0.03mL

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

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

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

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

REFERENCE

1. Hengartner, H., and Zuber, H.; FEBS *Lett.*, **37**, 212 (1973)
2. Kamei, S., Tomita, K., Nagata, K., Okuno, H., Shiraishi, T., Motoyama, A., Ohkubo, A., and Yamanaka, H.; *J. Clin. Biochem. Nutr.*, **3**,1 (1987)
3. Tomita, K., Kamei, S., Nagata, K., Okuno, H., Shiraishi, T., Motoyama, A., Ohkubo, A., and

Yamanaka, M.; *ibid*., **3**, 11 (1987)

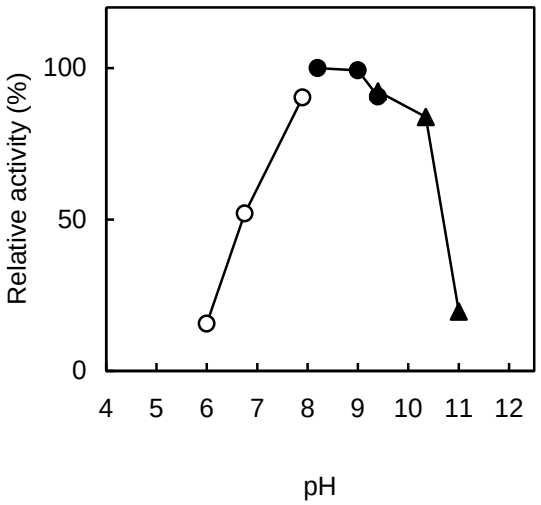

Fig. 1 pH profile

○ phosphate, ● Tris-HCl,
▲ carbonate

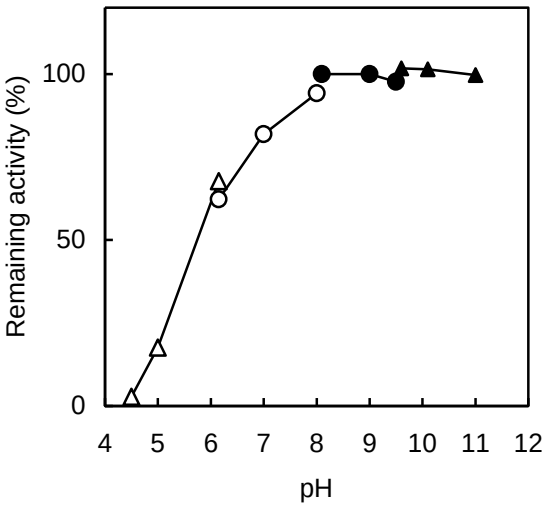

Fig. 2 pH stability

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

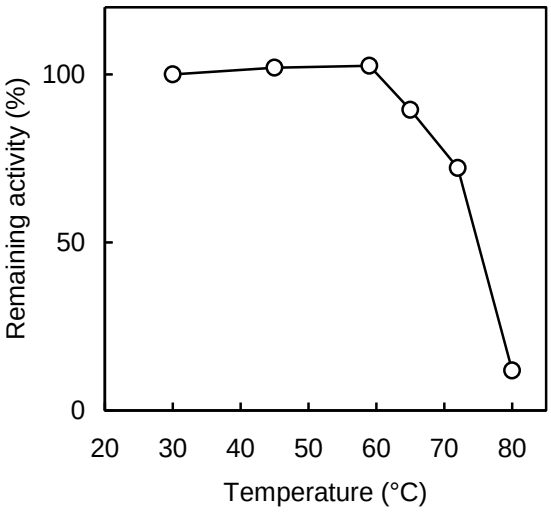

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tris-HCl buffer, pH 8.9

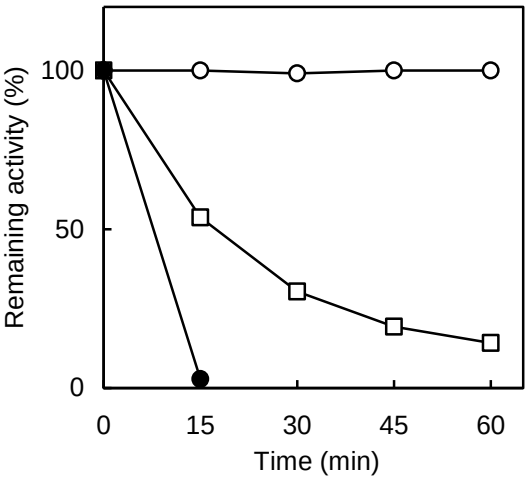

Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 8.9
○ 60 °C, □ 70 °C, ● 80 °C

NIPRO ENZYMES

α-GLUCOSIDASE (α-Glu)

[EC 3.2.1.20]

from *Bacillus stearothermophilus*

α-D-Glucoside + H_2O ↔ D-Glucose + Alcohol

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 40 U/mg protein	
Contaminants	:	(as α-Glu activity = 100 %)	
		Phosphoglucomutase	< 0.01 %
		NADH oxidase	< 0.01 %
		Alcohol dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 50,000	
Optimum pH	:	6.0 - 7.0	(Fig. 1)
pH stability	:	5.0 - 11.0	(Fig. 2)
Isoelectric point	:		
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(50 mM Potassium phosphate buffer, pH 6.3, at 30 °C)	
		p-Nitrophenyl-α-glucopyranoside (PNPG)	0.73 mM
		Maltose	1.3 mM
		Phenyl-α-glucopyranoside	2.4 mM
Substrate specificity	:	PNPG	100 %
		Maltose	177 %
		Phenyl-α-glucopyranoside	59 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, α-amylase determination.

ASSAY

Principle

The change in absorbance is measured at 400 nm according to the following reaction.

p-Nitrophenyl-α-glucopyranoside (PNPG) $\xrightarrow{\text{α-Glu}}$ p-Nitrophenol (PNP) + Glucose

Unit Definition

One unit of activity is defined as the amount of α-Glu that forms 1 μmol of PNP per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Potassium phosphate buffer, pH 6.3

Ⅱ PNPG solution ; 20 mM (0.603 g PNPG/100 mL distilled water) (Stable for two weeks if stored at 0 - 5 °C)

Ⅲ Na_2CO_3 solution ; 0.2 M (2.12 g Na_2CO_3/100 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 0.006 to 0.022 U/mL with 10 mM Potassium phosphate buffer containing 1 mg/mL BSA, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 1.5 mL of reaction mixture into a test tube.
   Solution Ⅰ 10.0mL
   Solution Ⅱ 5.0mL
2. Incubate at 30 °C for 5 minutes.
3. Add 0.5 mL of the enzyme solution and mix.
4. Incubate at 30 °C for exactly 15 minutes.
5. After incubation, add 2.0 mL of Solution Ⅲ and mix.
6. Read absorbance at 400 nm (Abs•test).
   At the same time, prepare the blank with 1.5 mL of the reaction mixture, and add 2.0 mL of Solution Ⅲ after incubation at 30 °C for 15 minutes, followed by addition of the enzyme solution (Abs•blank).

Calculation

$$\text{Volume activity (U/mL)} = ((\text{Abs•test}) - (\text{Abs•blank})) \times \frac{4.0}{18.1 \times 15 \times 0.5} \times \text{d.f.}$$

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

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

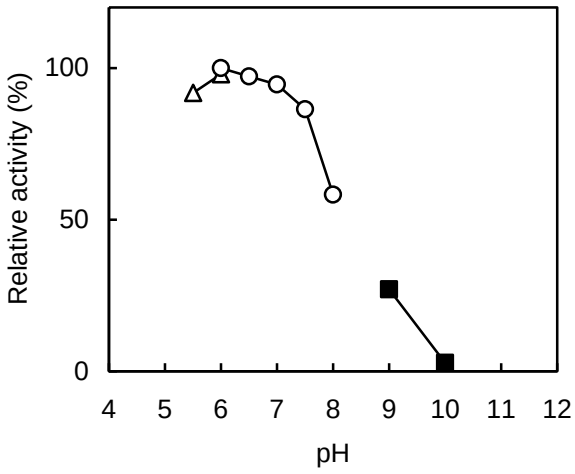

Fig. 1 pH profile

△ acetate, ○ phosphate,
■ Gly-NaOH

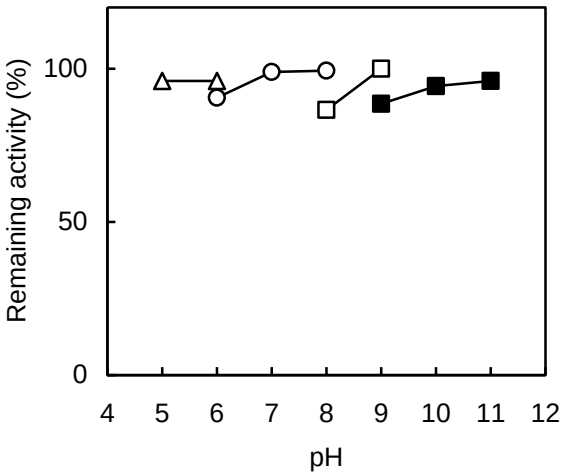

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
□ TEA-NaOH, ■ Gly-NaOH

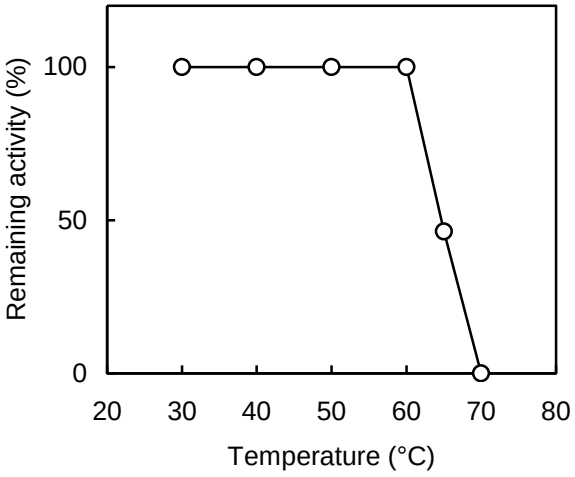

Fig. 3 Thermal stability

treated for 15 min in 0.1M potassium phosphate buffer, pH 8.0

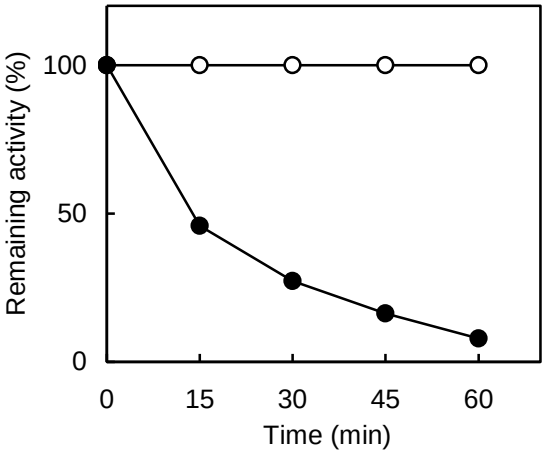

Fig. 4 Thermal stability

treated for in 0.1M potassium phosphate buffer, pH 8.0
○ 60 °C, ● 65°C

NIPRO ENZYMES

LEUCINE DEHYDROGENASE (LeuDH)

[EC 1. 4. 1. 9]

from *Bacillus stearothermophilus*

L-Leucine + NAD^+ + H_2O ↔ α-Ketoisocaproate+ NH_4^+ +NADH

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 40 U/mg protein	
Contaminants	:	(as LeuDH activity = 100 %)	
		NADH oxidase	< 0.01 %
		Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 300,000	
Subunit molecular weight	:	ca. 49,000	
Optimum pH	:	10.6	(Fig. 1)
pH stability	:	6.0 - 11.5	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(125mM Sodium phosphate buffer, pH 10.5, at 30 °C)	
		L-Leucine	3.4 mM
		NAD^+	0.3 mM
Substrate specificity	:	L-Leucine	100 %
		L-Valine	86 %
		L-Isoleucine	73 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of L-leucine, L-valine or L-isoleucine.

ASSAY

Principle

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

$$\text{L-Leucine} + NAD^+ + H_2O \xrightarrow{\text{LeuDH}} \alpha\text{-Ketoisocaproate} + NH_4^+ + NADH$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 250 mM Sodium phosphate, pH 10.5

Ⅱ L-Leucine solution ; 60 mM (0.787 g L-leucine/80 mL distilled water, adjusted to pH 10.5 with 1 N-NaOH and filled up to 100 mL with distilled water)

Ⅲ NAD^+ solution ; 100mM (0.663 g NAD^+/ 10mL with distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 100 mM sodium phosphate buffer, pH 9.5.

Procedure

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

Solution Ⅰ	15.00mL	Solution Ⅲ	0.93mL
Solution Ⅱ	10.00mL	H_2O	4.07mL

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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Ohshima, T., Nagata, S., and Soda, K.; *Arch. Microbiol*., **141**, 407 (1985)

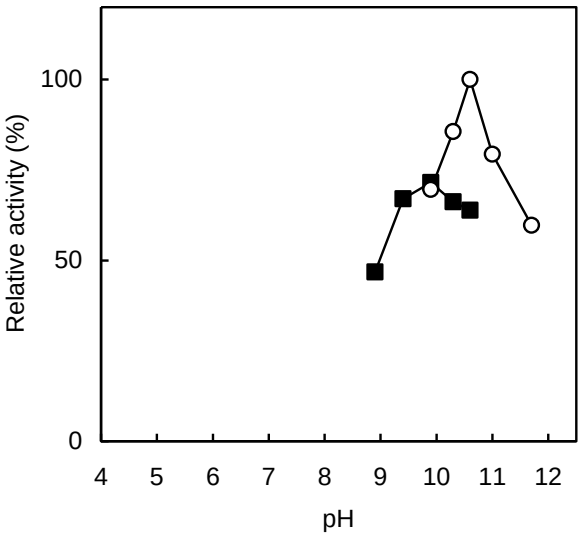

Fig. 1 pH profile

(■ Gly-KOH, ○ phosphate)

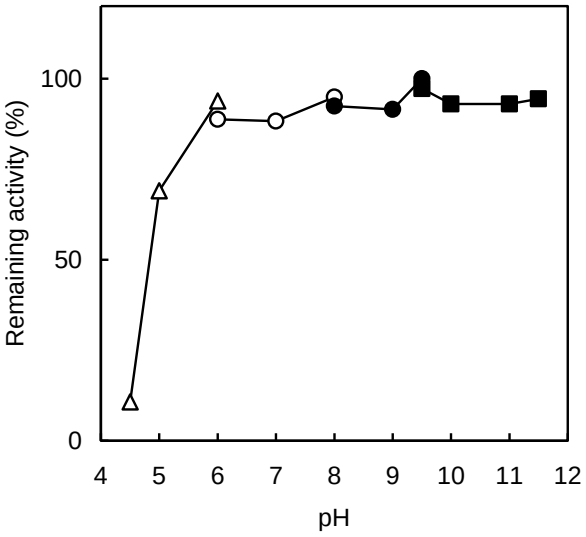

Fig. 2 pH stability

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

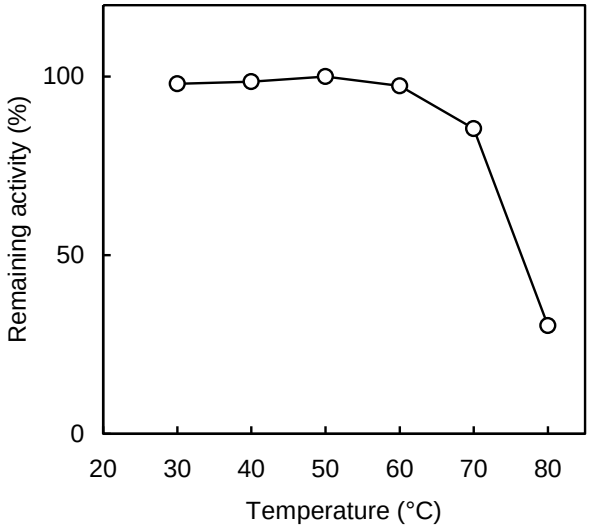

Fig. 3 Thermal stability

(treated for 15 min in 0.1M Gly-KOH buffer, pH 9.0)

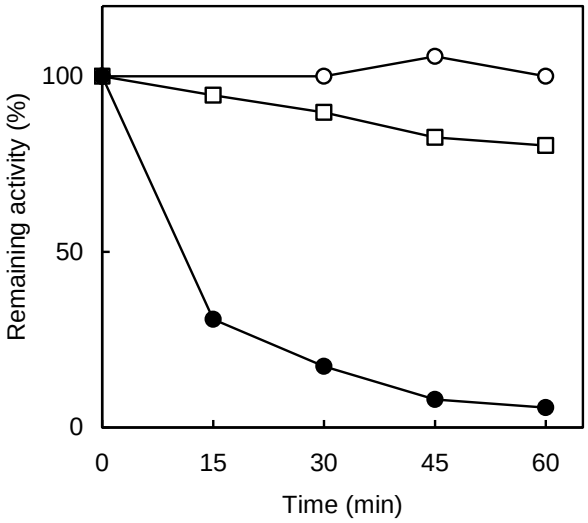

Fig. 4 Thermal stability

(treated in 0.1M Gly-KOH buffer, pH 9.0
○ 60°C, □ 70°C, ● 80°C)

NIPRO ENZYMES

PHOSPHOFRUCTOKINASE (PFK)

[EC 2. 7. 1. 11]

from *Bacillus stearothermophilus*

ATP + D-Fructose 6-phosphate ↔ ADP + D-Fructose 1, 6-bisphosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 100 U/mg protein	
Contaminants	:	(as PFK activity = 100 %)	
		Adenylate kinase	< 0.01 %
		ATPase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Glutathione reductase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		Glucose phosphate isomerase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 74,000	
Subunit molecular weight	:	ca. 34,000	
Optimum pH	:	9.0	(Fig. 1)
pH stability	:	6.5 - 10.0	(Fig. 2)
Isoelectric point	:	6.0 - 6.2	
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	:	(91mM Tris-HCl buffer, pH 9.0, at 30 °C)	
		Fructose 6-phosphate	1.6 mM
		ATP	0.035 mM
Activators	:	K^+, $(NH_4)_2SO_4$	
Inhibitors	:	PEP, Citrate	

STORAGE

Stable at -20 °C for at least one year

ASSAY

Principle

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

Fructose 6-phosphate + ATP $\xrightarrow{PFK}$ Fructose 1, 6-bisphosphate + ADP

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

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

Unit Definition

One unit of activity is defined as the amount of PFK that forms 1 μmol of fructose 1, 6-bisphosphate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCI, pH 9.0
Ⅱ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅲ Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
Ⅳ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10mL distilled water)
Ⅴ Fructose 6-phosphate (F6P) solution ; 500 mM (1.55 g F6P disodium salt/10 mL distilled water)
Ⅵ KCI solution ; 2.5 M (16.64g KCl/100 mL distilled water)
Ⅶ $MgSO_4$ solution ; 100 mM (2.47 g $MgSO_4 \cdot 7H_2O$/100 mL distilled water)
Ⅷ Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C
Ⅸ 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 50mM potassium phosphate buffer, pH 8.0.

Procedure

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

Solution Ⅰ	27.33mL	SolutionⅥ	0.06mL
Solution Ⅱ	0.30mL	SolutionⅦ	0.60mL
Solution Ⅲ	0.39mL	SolutionⅧ	0.06mL
SolutionⅣ	0.60mL	SolutionⅨ	0.06mL
SolutionⅤ	0.60mL		

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. Hengartner, H., and Harris, J.I.; FEBS *Lett*., **55**, 282 (1975)

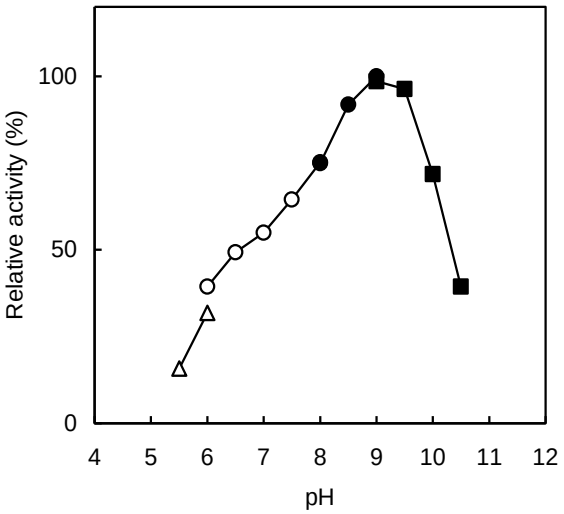

Fig. 1 pH profile

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

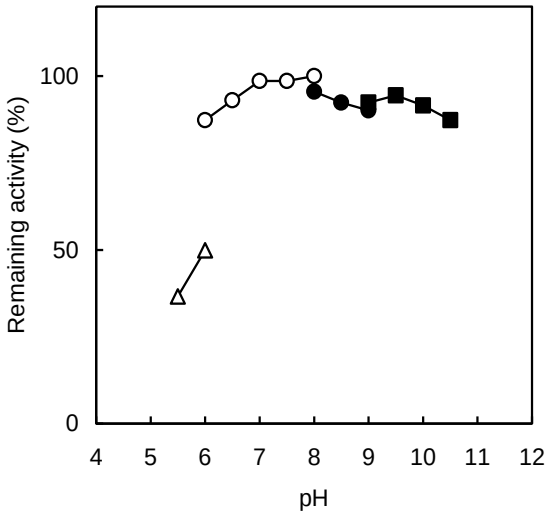

Fig. 2 pH stability

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

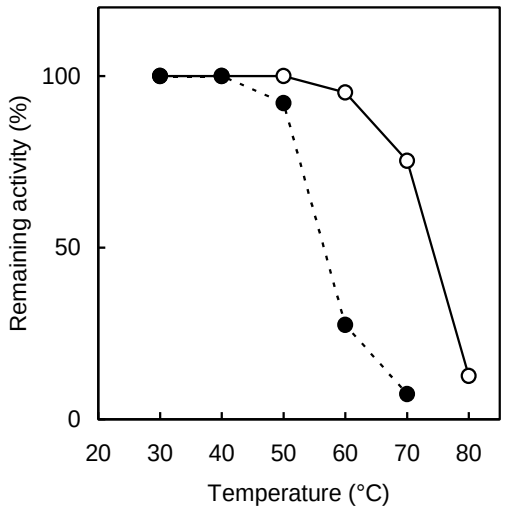

Fig. 3 Thermal stability

treated for 15 min in 50 mM Tris-HCl buffer, pH 8.5, or potassium phosphate buffer, pH7.5
○ phosphate, ● Tris-HCl

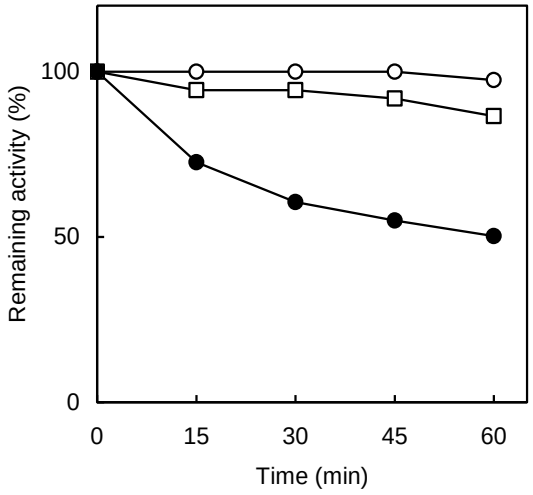

Fig. 4 Thermal stability

treated in 50 mM potassium phosphate buffer, pH 7.5
○ 50 °C, □ 60 °C, ● 70 °C

NIPRO ENZYMES

PHOSPHOGLUCOSE ISOMERASE (PGI)

[EC 5. 3. 1. 9]

from *Bacillus stearothermophilus*

D-Glucose 6-phosphate ↔ D-Fructose 6-phosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 400 U/mg protein	
Contaminants	:	(as PGI activity = 100 %)	
		Phosphofructokinase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		NADPH oxidase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 200,000	
Subunit molecular weight	:	ca. 54,000	
Optimum pH	:	9.0 - 10.0	(Fig. 1)
pH stability	:	6.0 - 10.5	(Fig. 2)
Isoelectric point	:	4.2	
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(95mM Tris-HCI buffer, pH 9.0, at 30 °C)	
		Fructose 6-phospate	0.27 mM

STORAGE

Stable at -20 °C for at least one year

ASSAY

Principle

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

$$\text{Fructose 6-phosphate} \xrightarrow{\text{PGI}} \text{Glucose 6-phosphate}$$

$$\text{Glucose 6-phosphate} + NADP^+ \xrightarrow{\text{G6PDH}} \text{Gluconolactone 6-phosphate} + NADPH + H^+$$

Unit Definition

One unit of activity is defined as the amount of PGI that forms 1 μmol of glucose 6-phosphate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 9.0
Ⅱ Fructose 6-phosphate (F6P) solution ; 100 mM (0.310 g F6P disodium salt/10 mL distilled water)
Ⅲ $NADP^+$ solution ; 22.5 mM (0.188 g $NADP^+$ sodium salt·$4H_2O$/10 mL distilled water)
Ⅳ Glucose-6-phosphate dehydrogenase (G6PDH) ; (from yeast, Roche Diagnostics K.K., No. 127 671) suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/2 mL) approx. 140 U/mg at 25 °C

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50mM 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	28.44 mL	Solution Ⅲ	0.60 mL
Solution Ⅱ	0.90 mL	Solution Ⅳ	0.06 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 the curve.

Calculation

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

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

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

REFERENCE

1. Muramatsu, N., and Nosoh, T.; *Arch. Biochem. Biophys*., **144**, 245 (1971)

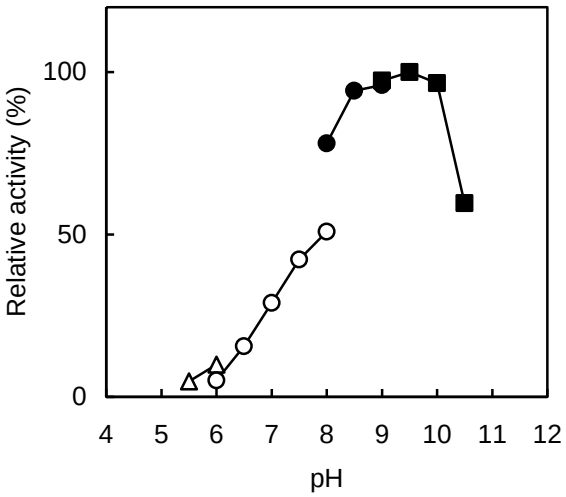

Fig. 1 pH profile

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

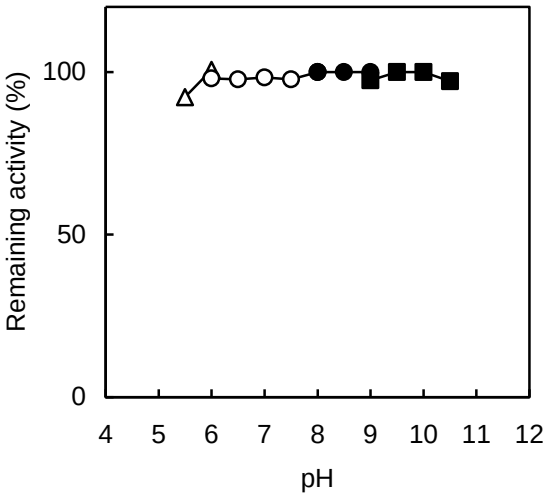

Fig. 2 pH stability

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

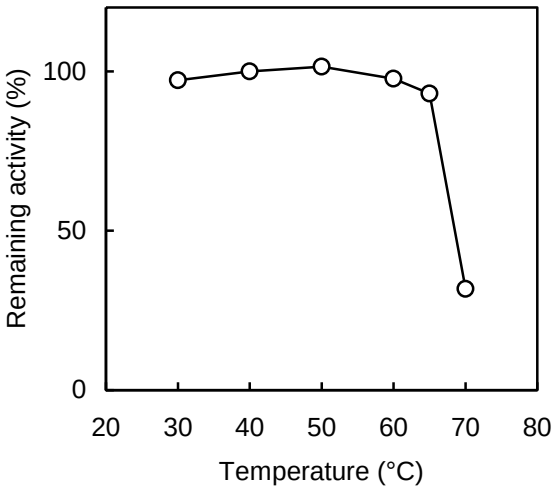

Fig. 3 Thermal stability

treated for 15 min in 50 mM Tris-HCl buffer, pH 8.5

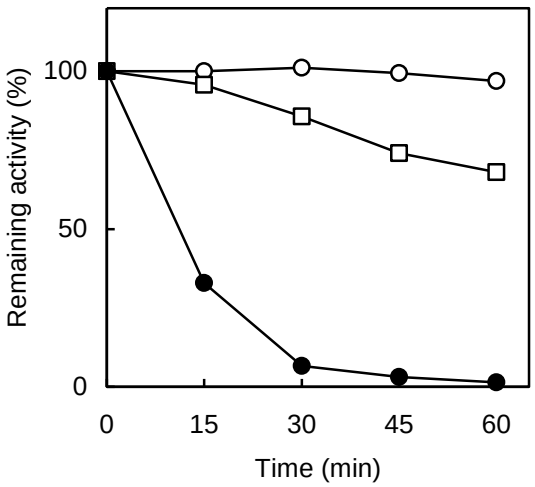

Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.5
○ 60 °C, □ 65 °C, ● 70 °C

NIPRO ENZYMES

PHOSPHOTRANSACETYLASE (PTA)

[EC 2. 3. 1. 8]

from *Bacillus stearothermophilus*

Acetyl-CoA + Pi ↔ Acetylphosphate + 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	:	(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.

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

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	26.0mL	Solution Ⅲ	1.0mL
Solution Ⅱ	2.0mL	Solution Ⅳ	1.0mL

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

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

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

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

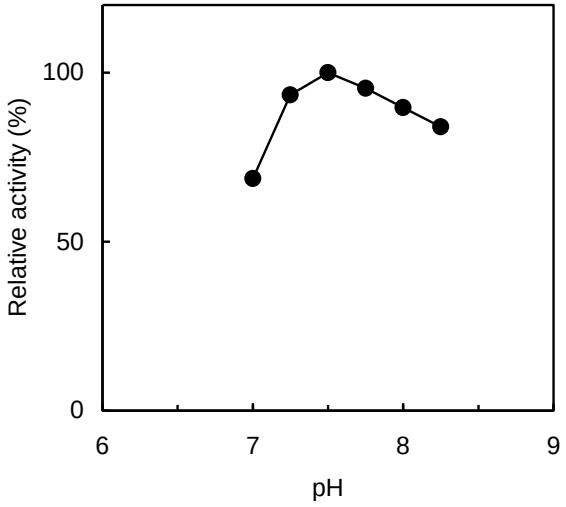

Fig. 1 pH profile

● Tris-HCl

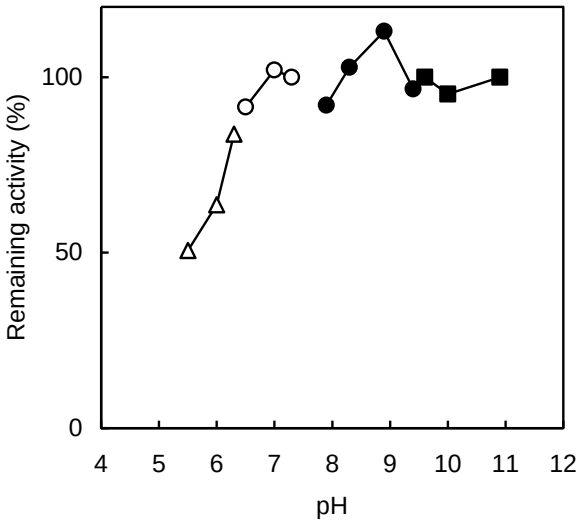

Fig. 2 pH stability

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

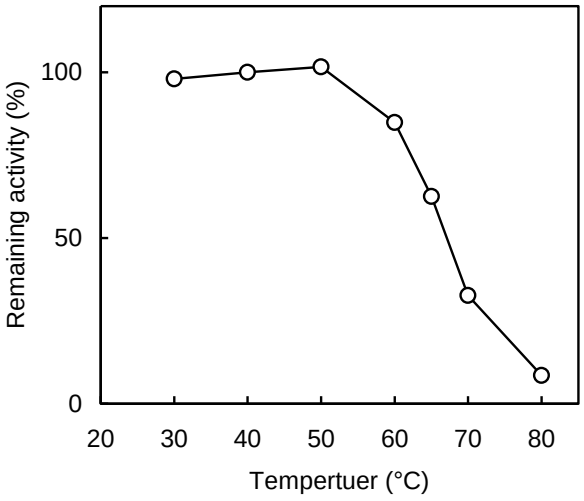

Fig. 3 Thermal stability

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

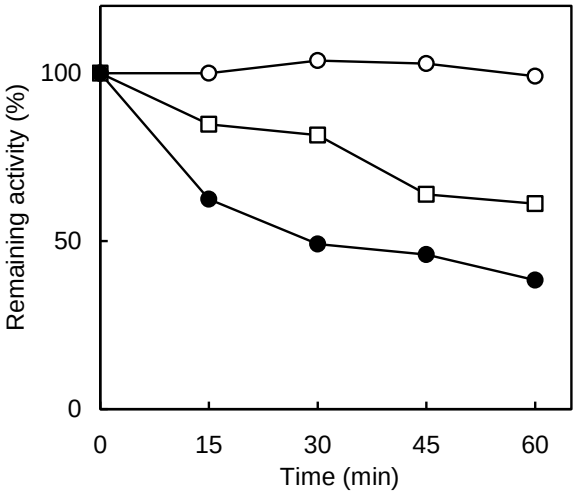

Fig. 4 Thermal stability

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

NIPRO ENZYMES

POLYNUCLEOTIDE PHOSPHORYLASE (PNPase)

[EC 2. 7. 7. 8]

from *Bacillus stearothermophilus*

RNA_{n+1} + Pi ↔ RNA_n + Nucleoside diphosphate

FOR DEPOLYMERIZATION REACTION

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 2,000 U/mg protein	

PROPERTIES

Molecular weight	: 300,000 - 340,000	
Subunit molecular weight	: ca. 85,000	
Optimum pH	: 9.0 - 9.5	(Fig. 1)
pH stability	: 9.0 - 11.0	(Fig. 2)
Isoelectric point	: 4.0	
Thermal stability	: No detectable decrease in activity up to 55 °C.	(Fig. 3, 4)
Michaelis constants	: (38 mM Tris-HCI buffer, pH 9.5, at 60 °C)	
	Poly A	0.27 mM**
	KH_2PO_4	3.0 mM
	**concentration of poly A was calculated as AMP concentration	
Effectors	: cations and anions	(Fig. 5, 6)

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for the preparation of polyribonucleotide.

ASSAY

Principle

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

$$\text{Poly } A_n + Pi \xrightarrow{\text{PNPase}} \text{Poly } A_{n-1} + ADP \qquad (I)$$

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

$$Pyruvate + NADH + H^+ \xrightarrow{LDH} Lactate + NAD^+ \qquad (II)$$

Unit Definition

One unit of activity is defined as the amount of PNPase that forms 1 µmol of ADP per hour at 60 °C by depolymerizing of Poly A.

Solutions

(Reaction I)

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 9.5 ((1.212 g Tris + 0.074 g EDTA + 0.014 mL 2-mercaptoethanol + 0.610 g $MgCl_2 \cdot 6H_2O$ + 0.746 g KCl)/80 mL distilled water, adjusted to pH 9.5 with 1 N-HCl and filled up to 100 mL with distilled water)

Ⅱ KH_2PO_4 solution ; 65 mM (0.088 g KH_2PO_4/10 mL distilled water)

Ⅲ polyadenylate (Poly A) solution ; (25 mg Poly A potassium salt/1 mL distilled water; ca. 35 mM based on AMP concentration)

(Reaction II)

Ⅳ Buffer solution ; 100 mM Triethanolamine buffer, pH 7.6 ((9.300 g triethanolamine-HCl + 0.407 g $MgCl_2 \cdot 6H_2O$ + 0.373 g KCl)/400 mL distilled water, adjusted to pH 7.6 with 1 N-NaOH and filled up to 500 mL with distilled water)

Ⅴ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)

Ⅵ Phosphoenolpyruvate (PEP) solution ; 56mM (0.150 g PEP MCA salt/10 mL distilled water)

Ⅶ Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C

Ⅷ 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 1 to 5 U/mL with 50 mM Tris-HCl buffer, pH 8.5.

Procedure

(Reaction I)

1. Prepare the following reaction mixture and pipette 0.55 mL of reaction mixture into a test tube.

Solution Ⅰ	2.50mL	Solution Ⅲ	1.00mL
Solution Ⅱ	1.00mL	H_2O	1.00mL

2. Add 0.10 mL of enzyme solution and mix.
3. Incubate at 60 °C for exactly 10 minutes.
4. After incubation, add 0.01 mL conc. HCl and mix.
5. Centrifuge at 10,000 rpm for 30 seconds.
   At the same time, repeat the Procedure 1 to 5 using distilled water in place of enzyme solution in Procedure 2 (as blank).

(Reaction II)

6. Prepare the following reaction mixture and pipette 2.50 mL of the reaction mixture into a cuvette.

Solution Ⅳ	24.18mL	Solution Ⅶ	0.12mL

Solution Ⅴ	0.40mL	Solution Ⅷ	0.05mL
Solution Ⅵ	0.25mL		

7. Incubate at 30 °C for about 3 minutes.
8. Add 0.10 mL of supernatant of Procedure 5 and mix.
9. Read absorbance at 340 nm (Abs•test).
   Repeat the Procedure using blank (Abs•blank).

Calculation

$$\text{Volume activity (U/mL)} = ((\text{Abs•blank}) - (\text{Abs•test})) \times \frac{2.60 \times 0.65}{6.22 \times 0.10 \times 0.10} \times \frac{60}{10} \times \text{d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
*Protein concentration ; determined by the absorbance at 280nm (Abs280), where 1 Abs280 = 1 mg/mL

REFERENCES

1. Smith, J.C., and Eaton, M.A.W.; *Nucleic Acids Research*, **1**, 1763 (1974)
2. Wood, J.N., and Hutchinson, D.W.; *ibid*., **3**, 219 (1976)

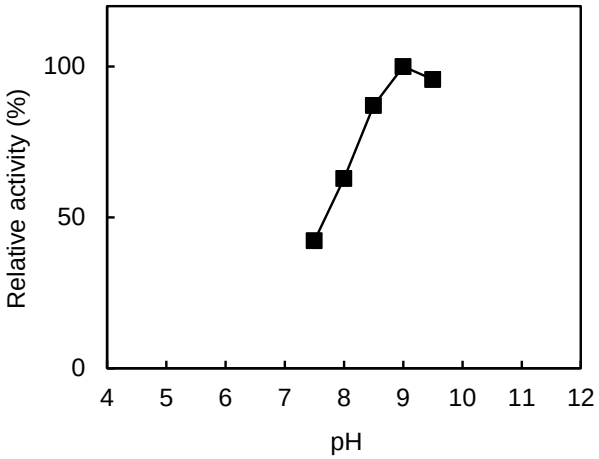

Fig. 1 pH profile

■ Tris-HCl

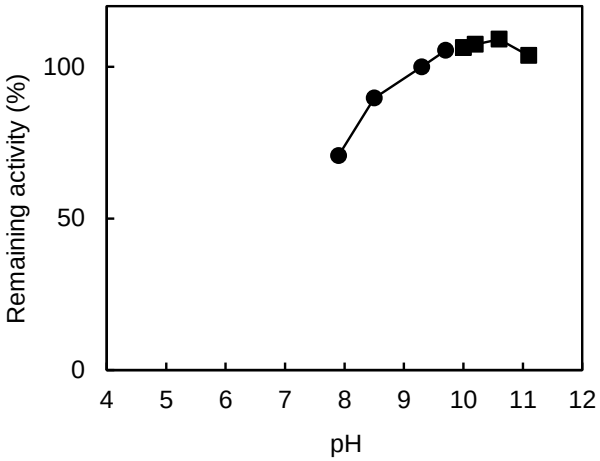

Fig. 2 pH stability

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

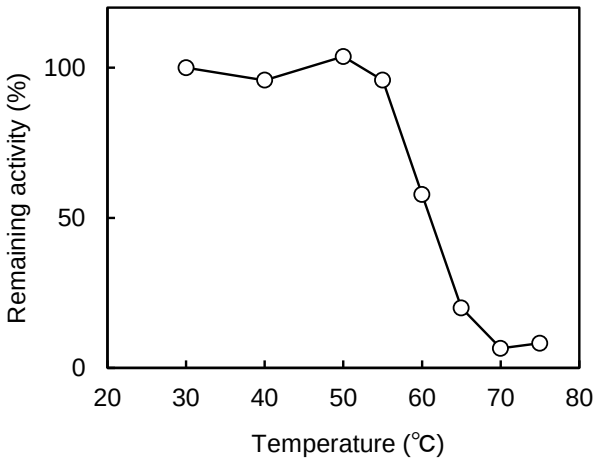

Fig. 3 Thermal stability

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

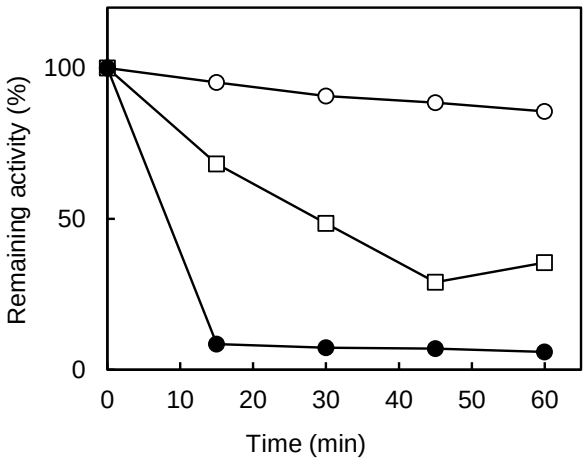

Fig. 4 Thermal stability

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

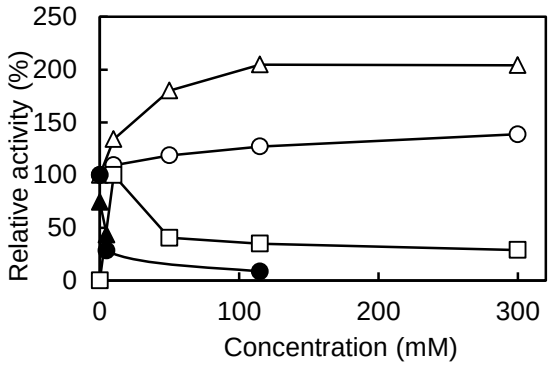

Fig. 5 Effect of various cations on the activity of Polynucleotide phosphorylase in the following *Assay Method*

Measurement : 0.015 mL of each cation solution, 0.010 mL of enzyme solution and 0.055 mL of reaction mixture were mixed, and reacted at 60 ° C. After 10 minutes, the quantity of ADP was determined.
○ NaCl, △ KCl, □ $MgCl_2$, ● $CaCl_2$, ▲$ZnCl_2$

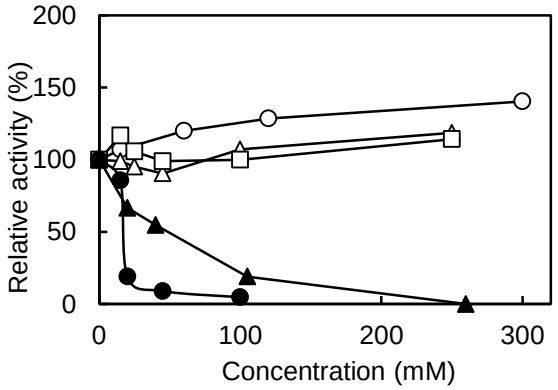

Fig. 6 Effect of various anions on the activity of Polynucleotide phosphorylase in the following *Assay Method*

Measurement : 0.015 mL of each anion solution, 0.010 mL of enzyme solution and 0.055 mL of reaction mixture were mixed, and reacted at 60 ° C. After 10 minutes, the quantity of ADP was determined.
○ NaCl, △CH_3COONa, □ Na_2SO_4, ●$NaHCO_3$, ▲ NaH_2PO_4

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.

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

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

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 ; 1.0 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	22.71mL	Solution Ⅴ	0.48mL
Solution Ⅱ	2.40mL	Solution Ⅵ	0.90mL
Solution Ⅲ	0.45mL	Solution Ⅶ	0.06mL
Solution Ⅳ	3.00mL		

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)

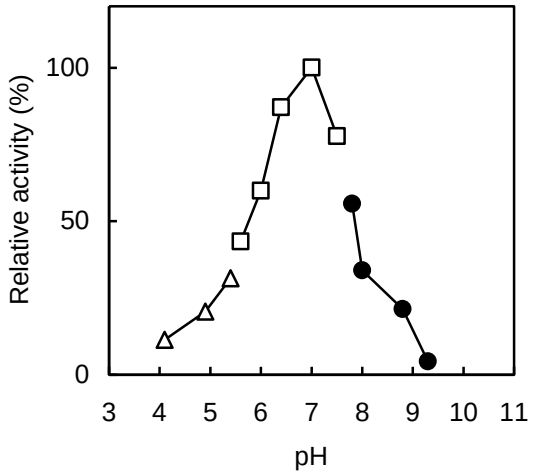

Fig. 1 pH profile

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

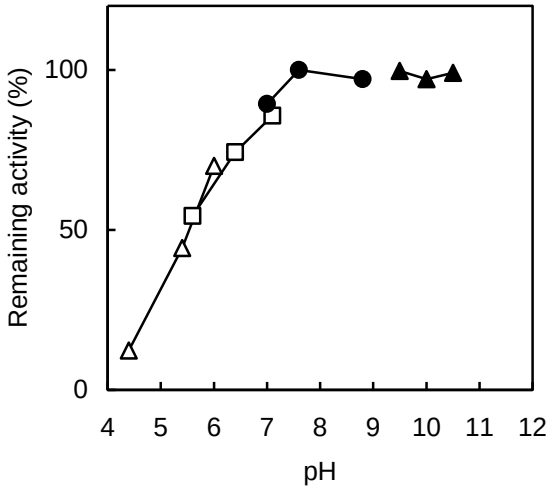

Fig. 2 pH stability

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

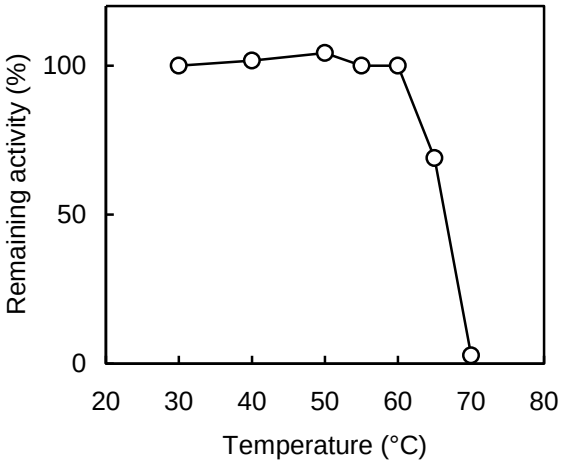

Fig. 3 Thermal stability

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

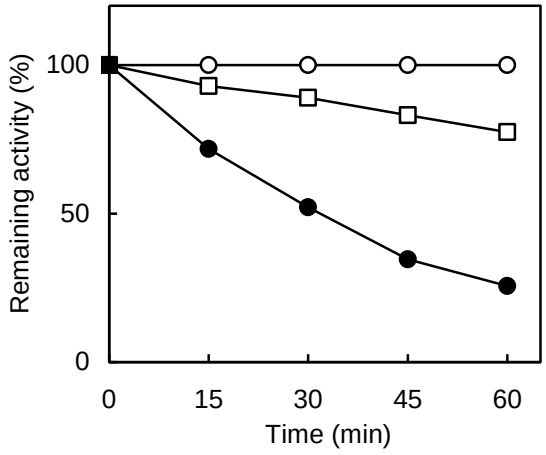

Fig. 4 Thermal stability

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

NIPRO ENZYMES

SUPEROXIDE DISMUTASE (SOD)

[EC 1.15.1.1]

from *Bacillus stearothermophilus*

$$O_2^- + O_2^- + 2H^+ \leftrightarrow O_2 + H_2O_2$$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 9,000 U/mg protein	
Contaminants	:	(as SOD activity = 100 %)	
		Catalase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 50,000	
Subunit molecular weight	:	ca. 25,000	
Metal content	:	1.5 g atoms of Mn per mole of enzyme	
Optimum pH	:	9.5	(Fig. 1)
pH stability	:	6.0 - 9.0	(Fig. 2)
Isoelectric point	:	4.5	
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for medicine, cosmetic material and nutrition or antioxidant.

ASSAY

Principle

To determine the enzyme activity of cytochrome c reduction is measured by the following reactions.

$$\text{Xanthine} + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Urate} + O_2^- + H_2O_2$$

$$O_2^- \xrightarrow{\text{cytochrome c}} \text{cytochrome c (red.)}$$

$$O_2^- \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

Unit Definition

One unit of activity is defined as the amount of SOD required to inhibit the rate of reduction of cytochrome C by 50 % at 30 °C.

Solutions

Ⅰ Buffer solution ; 75 mM Potassium phosphate buffer, pH 7.8

Ⅱ Xanthine solution ; 0.75 mM (0.010 g xanthine/50 mL N/250 NaOH)

Ⅲ Cytochrome c solution ; 0.15 mM (0.019 g cytochrome c/10 mL distilled water, Sigma-Aldrich Co., No. C-2506, from horse heart)

Ⅳ EDTA solution ; 1.5 mM (0.028 g EDTA disodium salt·$2H_2O$/50 mL distilled water)

Ⅴ Xanthine oxidase (XOD) ; (from buttermilk, Sigma-Aldrich Co., No. X-1875) suspension in 2.3 M $(NH_4)_2SO_4$ solution is diluted to 0.04 U/mL with distilled water. (prepare freshly)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to approx. 600 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.80 mL of reaction mixture and 0.005 mL of enzyme solution into a cuvette.

Solution Ⅰ	22.00mL	Solution Ⅲ	2.00mL
Solution Ⅱ	2.00mL	Solution Ⅳ	2.00mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.20 mL of Solution Ⅴ into the cuvette and mix.
4. Read absorbance change at 550 nm per minute for the linear portion of curve (ΔAbs•test)*.
5. Add 0.005 mL of Solution Ⅰ in place of enzyme solution and measure the same above 4 (ΔAbs•blank).

*Dilute enzyme solution with 50 mM potassium phosphate buffer, pH 7.5, because the decrease in the initial rate should not fall outside the range of 40 to 60 % for the results to be valid.

Calculation

$$\text{Volume activity (U/mL)} = \left[\frac{(\Delta\text{Abs•blank})}{(\Delta\text{Abs•test})} - 1 \right] \times \frac{601}{1} \times \text{d.f.}$$

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

d.f. ; dilution factor

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Bridgen, J., Harris, J.I., and Kolb, E.; *J. Mol. Biol.*, **105**, 333 (1976)
2. Brock, C.J., Harris, J.I., and Sato, S.; *ibid.*, **107**, 175 (1976)
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4. Auffret, A.D., Blake, T,J., and Williams, D.H.; *Eur. J. Biochem.*, **113**, 333 (1981)
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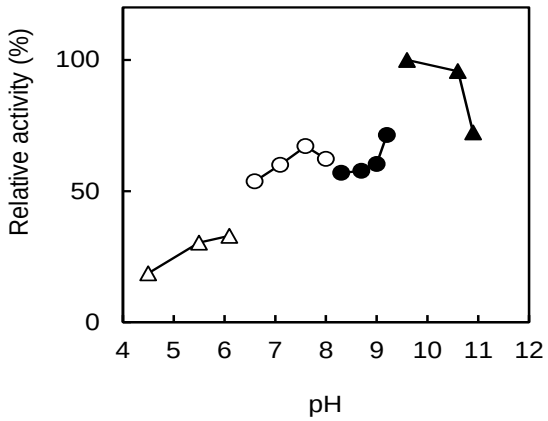

Fig. 1 pH profile

(△ acetate, ○ phosphate,)

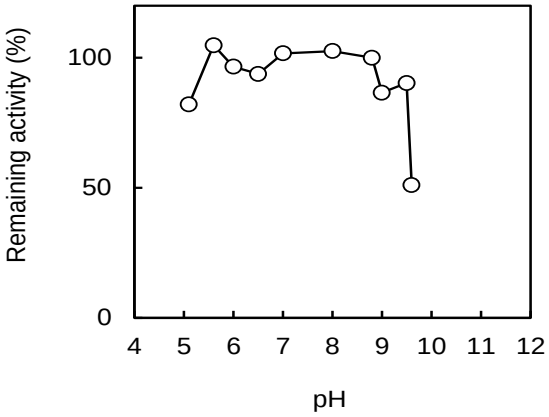

Fig. 2 pH stability

(treated for 24 hr at 4 ° C in the

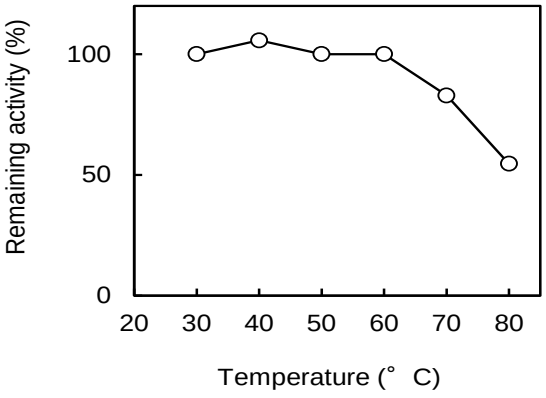

Fig. 3 Thermal stability

(treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5)

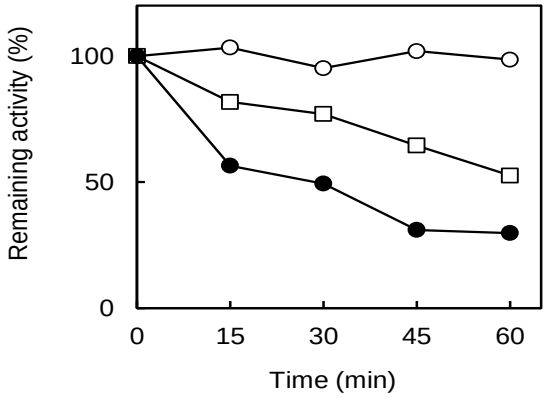

Fig. 4 Thermal stability

(treated in 0.1 M potassium phosphate buffer, pH 7.5)

NIPRO ENZYMES

BILIRUBIN OXIDASE (BOD3)

[EC 1.3.3.5]

from *Trachyderma tsunodae*

$$2\ \text{Bilirubin} + O_2 \rightarrow 2\ \text{Biliverdin} + 2\ H_2O$$

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 100 U/mg protein	

PROPERTIES

Molecular weight	: ca. 60,000	(SDS-electrophoresis)	
	: ca. 80,000	(Gel filtration)	
Optimum pH	: 5.0		(Fig. 1)
pH stability	: 4.0 – 11.0	(4 °C, 24 hr)	(Fig. 2)
Isoelectric point (calculation)	: 3.8		
Optimum temperature	: 65 – 80 °C		(Fig. 3)
Thermal stability	: No detectable decrease in activity up to 50 °C.	(pH 7.0)	(Fig. 4, 5)
Michaelis constants	: See table 1		
Substrate specificity	: See table 1		

STORAGE

Stable at -20 °C for one year

APPLICATION

The enzyme is useful for enzymatic determination of bilirubin.
It could be used as a cathode catalyst in biofuel cells.

ASSAY

Principle

The change in absorbance is measured at 500 nm according to the following reaction.

$$\text{Phenol} + \frac{1}{2}O_2 + H_2O \xrightarrow{\text{BOD3}} \text{Quinone and/or Phenoxy radical} + H_2O_2$$

$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\,H_2O$$

Unit Definition

One unit of activity is defined according to the calculation formula below.

Solutions

Ⅰ Buffer solution ; 300 mM Potassium phosphate buffer, pH7.0
Ⅱ 4-Aminoantipyrine (4-AA) solution ; 24.6 mM (0.25 g 4-AA / 50 mL distilled water)
Ⅲ Phenol solution ; 420 mM (1.98 g phenol/50mL distilled water)
Ⅳ Peroxidase*[1] (POD) solution ; 240 U/mL (2,400 U/10mL distilled water)
*[1]POD: TOYOBO Co., LTD. Grade Ⅲ #PEO-302

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 15 to 60 U/mL with 10 mM potassium phosphate buffer, pH 7.0 containing 0.1 % BSA.

Procedure

1. Prepare the following reaction mixture and pipette 0.90 mL of reaction mixture into a cuvette.

Solution Ⅰ	4.00 mL
Solution Ⅱ	0.40 mL
Solution Ⅲ	0.40 mL
Solution Ⅳ	0.40 mL
H_2O	6.40 mL

2. Incubate at 37 °C for about 3 minutes.
3. Add 0.005 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 500 nm per minute (ΔAbs (test)) in linear portion of curve. Repeat the procedure 3 using distilled water in place of enzyme solution, and ΔAbs (blank) is obtained.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta\text{Abs (test)} - \Delta\text{Abs (blank)}) \times (0.90 + 0.005)}{11.11 \times 0.005 \times 1/20} \times \text{d.f.}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)}^{*2}}$$

d.f. ; dilution factor
11.11 ; millimolar extinction coefficient of quinoneimine dye at 500 nm (cm^2/μmol)
1/20 ; coefficient of transformation for internal unit definition
*[2]Protein concentration ; determined by Bradford's method

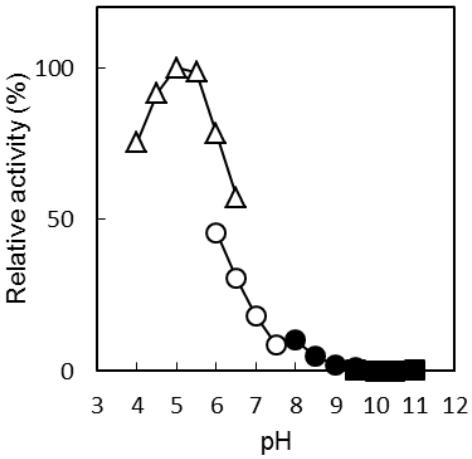

Fig. 1 pH profile

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

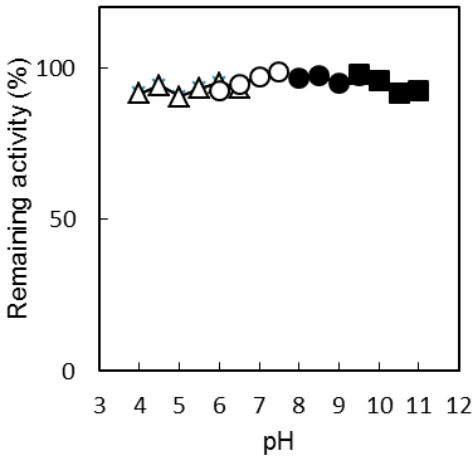

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (50 mM);
△ acetate, ○ phosphate
● Tris-HCl, ■ Glycine-KOH

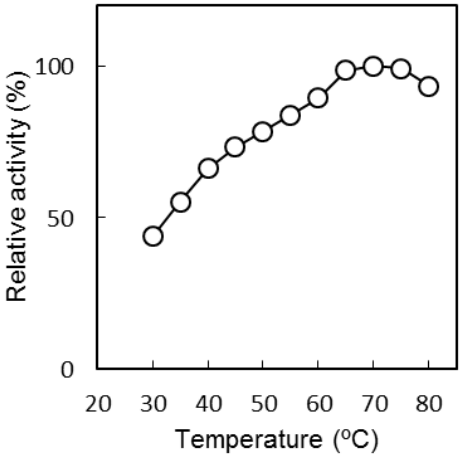

Fig. 3 Thermal activity

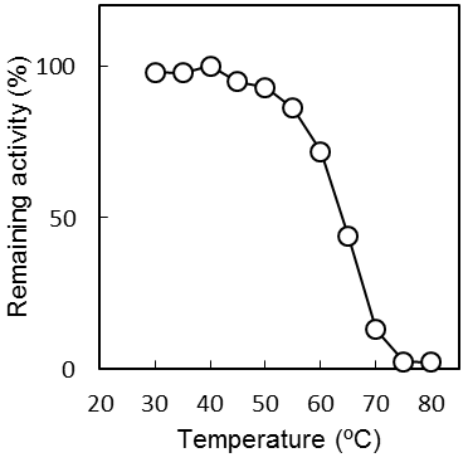

Fig. 4 Thermal stability

treated for 15 min in 20 mM potassium phosphate buffer, pH 7.0

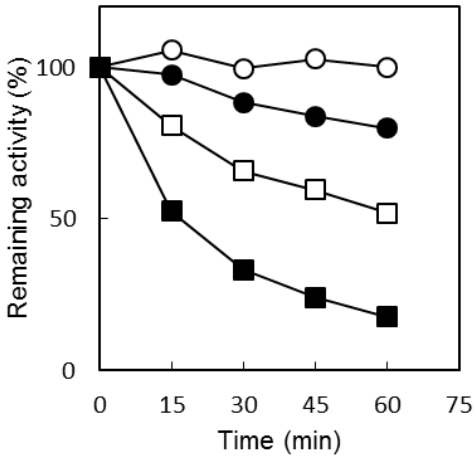

Fig. 5 Thermal stability

(treated in 20 mM potassium phosphate buffer, pH 7.0
○ 50 ºC, ● 55 ºC, □ 60 ºC, ■ 65 ºC)

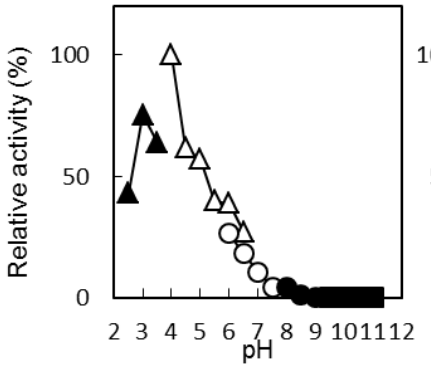

Fig. 6 pH profile (ABTS*3)

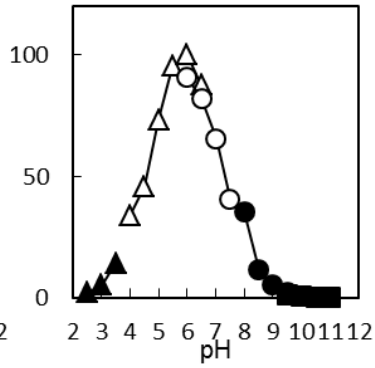

Fig. 7 pH profile (Bilirubin C*4)

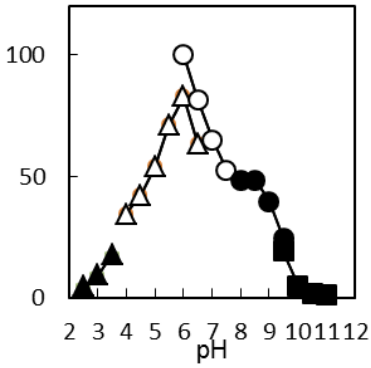

Fig. 8 pH profile (Bilirubin F*4)

(Measured in 20 mM buffer.
▲ Glycine-HCl, △ acetate, ○ phosphate, ● Tris-HCl, ■ Glycine-KOH)

*3 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic Acid) Diammonium Salt
*4 Bilirubin C (conjugated type) and Bilirubin F (free type) are from [Interference Check. A Plus] (Sysmex, Kobe, Japan).

Table 1. Substrate specificity of BOD3

	Phenol	ABTS	Bilirubin C	Bilirubin F
Optimum pH	5.0	4.0	6.0	6.0
Michaelis constants (μM)	41	39	26	26
Relative activity (%)	100	427	36	8
Wavelength for Measurement (nm)	500	405	450	450
Extinction Coefficient (cm^2/μmol)	11.11	29	74	32

Michaelis constants and activity of phenol were defined at pH 7.0. They were defined at each optimum pH when the substrate was ABTS, Bilirubin C, or Bilirubin F,.

NIPRO ENZYMES

DIAPHORASE 3 (DI-3)

[EC 1. 6. 99. -]

from recombinant *E. coli*

$$NAD(P)H + Acceptor(ox.) + H^{+} \leftrightarrow NAD(P)^{+} + Acceptor(red.)$$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 1,000 U/mg protein	
Contaminants	:	(as Diaphorase activity = 100 %)	
		Adenylate kinase	< 0.01 %
		NADH oxidase	< 0.01 %

PROPERTIES

Subunit molecular weight	:	ca. 20,000 (SDS-electrophoresis)	
Optimum pH	:	8.0	(Fig. 1)
pH stability	:	7.5 - 9.5	(Fig. 2)
Isoelectric point	:	4.7	
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	See Table 1	

STORAGE

Stable at -20 to 5 °C for one year

APPLICATION

The enzyme is useful for the measurement of various dehydrogenase reactions in visible spectral range.

ASSAY

Principle

The change in absorbance is measured at 600 nm according to the following reaction.

$$NAD(P)H + DCIP(ox.) + H^+ \xrightarrow{DI\text{-}3} NAD(P)^+ + DCIP(red.)$$

Unit Definition

One unit of activity is defined as the amount of DI-3 that reduces 1 μmol of DCIP per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 500 mM Tris-HCl, pH8.5

Ⅱ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)

Ⅲ 2,6-Dichlorophenolindophenol (DCIP) solution ; 1.2 mM (2.0 mg DCIP sodium salt·$2H_2O$/5mL distilled water) (prepare freshly)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 1.0 to 2.0 U/mL with 50 mM potassium phosphate buffer, pH 7.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.28 mL of reaction mixture and 0.12 mL of Solution Ⅲ into a cuvette.

Solution Ⅰ	3.00mL
Solution Ⅱ	2.28mL
H_2O	23.22mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.008 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 600 nm per minute (ΔAbs(test)) in linear portion of curve. Repeat the Procedure 3 using distilled water in place of enzyme solution, and ΔAbs(blank) is obtained.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs\ (test) - \Delta Abs\ (blank)) \times (2.40 + 0.008)}{19 \times 0.008} \times d.f.$$

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

d.f. ; dilution factor

19 ; millimolar extinction coefficient of DCIP (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Mains, I., Power, D.M., Thomas, E.W. and Buswell J. A.; *Biochem. J.*, **191**, 457 (1980)

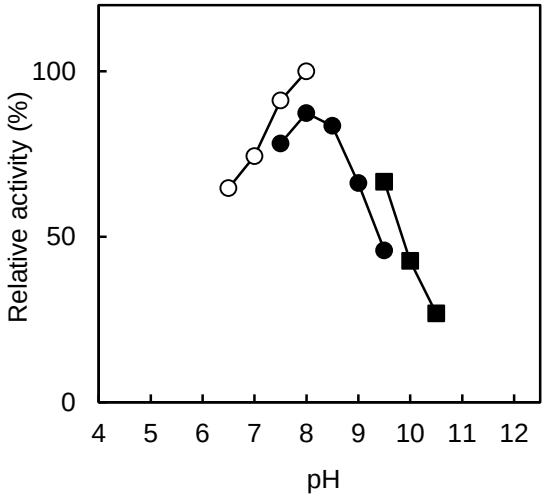

Fig. 1 pH profile

○ phosphate, ● Tris-HCl,
■ Gly-KCl-KOH

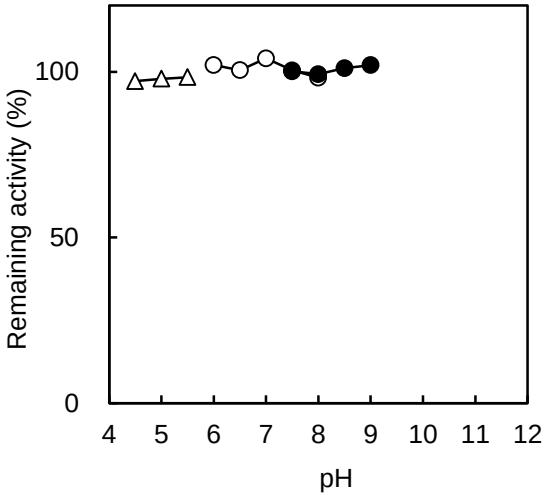

Fig. 2 pH stability

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

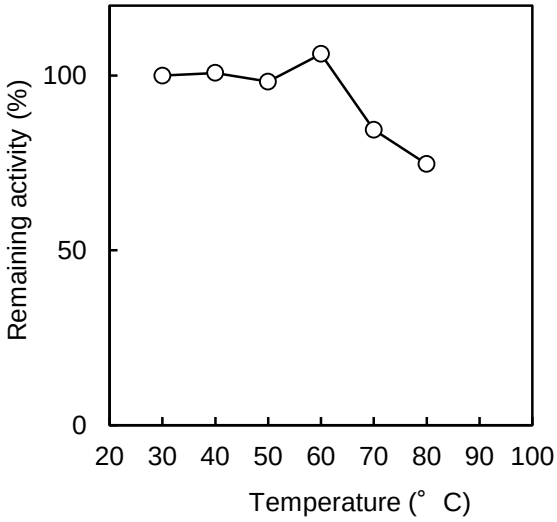

Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

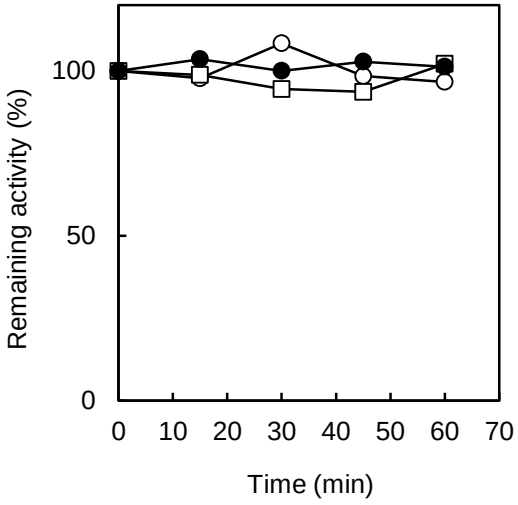

Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.5
○ 50 ℃, □ 60 ℃, ● 70 ℃

NIPRO ENZYMES

Table 1. SUBSTRATE SPECIFICITY OF DIAPHORASE

Acceptor	DCIP[*1]	NTB[*2]	MTT[*3]
$Km^{Acceptor}$ (mM)	0.02	0.15	0.9
Km^{NADH} (mM)	0.37	0.01	0.05
Km^{NADPH} (mM)	32.7	0.31	2.0
Optimum pH	8.0	10	8.0
Assay Mixture	Tris-HCl (pH 8.5) 50 mM NAD(P)H 1 mM DCIP 0.06 mM	TEA (pH 7.0) 50 mM NAD(P)H 1 mM NBT 0.5 mM Triton X-100 0.1 %	TEA (pH 7.0) 50 mM NAD(P)H 1 mM MTT 0.5 mM Triton X-100 0.5 %
Wavelength for Measurement (nm)	600	550	565
Extinction Coefficient ($cm^2/\mu mol$)	19	12.4	20

*1 2,6-Dichlorophenolindophenol
*2 Nitrotetrazolium Blue
*3 Thiazolyl Blue Tetrazolium Bromide

pH profiles of DI-3 (Acceptor; NTB or MTT)

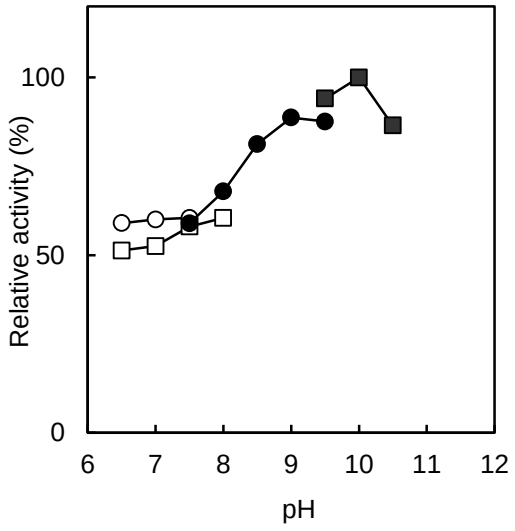

Fig. 5 pH profile (NTB)

□ triethanolamine, ○ phosphate,
● Tris-HCl, ■ Gly-KCl-KOH

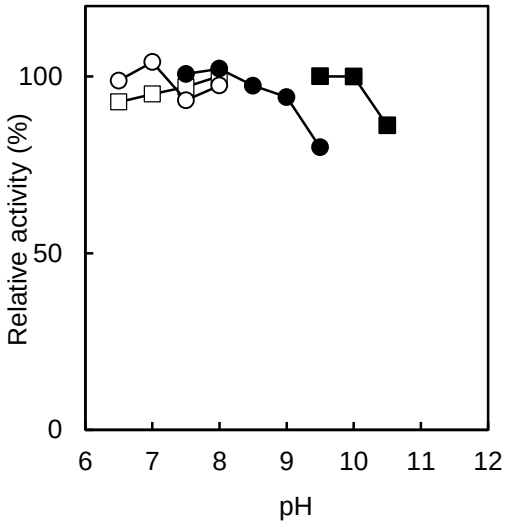

Fig. 6 pH profile (MTT)

□ triethanolamine, ○ phosphate,
● Tris-HCl, ■ Gly-KCl-KOH

NIPRO ENZYMES

DIAPHORASE 22 (Di-22)

[EC 1. 8. 1. 4]

from recombinant *E.coli*

$NADH + Acceptor(ox.) + H^+ \leftrightarrow NAD^+ + Acceptor(red.)$

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 150 U/mg protein	
Contaminants	: (as Diaphorase activity = 100 %)	
	Adenylate kinase	< 0.01 %
	NADH oxidase	< 0.20 %

PROPERTIES

Molecular weight	: ca. 110,000	
Subunit molecular weight	: ca. 50,000	
Optimum pH	: 8.0	(Fig,1)
pH stability	: 6.0 – 9.0	(Fig.2)
Thermal stability	: No detectable decrease in activity up to 70 °C.	(Fig. 3, 4)
Michaelis constants	: (50 mM HEPES buffer, pH 7.0, at 30 °C)	
	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT)	0.345 mM
	NADH	0.033 mM
		(Table 1)
Substrate specificity	: NADH	100 %
	NADPH	1 %
	MTT	100 %
	Lipoate	103 %
		(Table 1)

STORAGE

Store at -20℃

APPLICATION

The enzyme is useful for measurement of various dehydrogenase reactions in the visible spectral range.

ASSAY

Principle

The change in absorbance is measured at 565 nm according to the following reaction.

$$NADH + MTT(ox.) + H^+ \xrightarrow{\text{Di-22}} NAD^+ + MTT(red.)$$

Unit Definition

One unit of activity is defined as the amount of Diaphorase that forms 1 μmol of NAD^+ per minute at 30 °C

Solutions

Ⅰ Buffer solution ; 100 mM HEPES, pH 7.0

Ⅱ 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT) solution ; 10 mM (20 mg MTT disodium salt·$2H_2O$/5 mL distilled water)

Ⅲ NADH solution ; 13.1mM (0.100g NADH disodium salt·$3H_2O$ /10 mL distilled water)

Ⅳ Triton solution ; 10 % (1 mL TritonX-100 dilute with distilled water up to10 mL)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 1 to 5 U/mL with 50 mM potassium phosphate buffer, pH 7.5, 1mg/mL BSA.

Procedure

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

Solution Ⅰ	15.00mL	SolutionⅣ	1.50mL
Solution Ⅱ	1.50mL	H_2O	10.80mL
Solution Ⅲ	1.20mL		

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 565 nm per minute (ΔAbs_{565}) in the linear portion of curve.

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{565}) \text{ X } (3.00 + 0.01)}{20.0 \text{ X } 0.01} \text{ X d.f.}$$

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

d.f. ; dilution factor

20.0 ; millimolar extinction coefficient of MTT (cm^2/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Packman, L.C., and Perham. R.N.; FEBS *Lett*., **139**, 155 (1982)

Table 1. SUBSTRATE SPECIFICITY OF DIAPHORASE 22

Acceptor	MTT	Lipoate
$Km^{Acceptor}$ (mM) Km^{NADH} (mM)	0.345 0.033	2.0 0.01
Relative Activity	100	103
Assay Mixture	HEPESI (pH 7.0) 50 mM NADH 0.5 mM MTT 0.5 mM Triton X-100 0.5 %	Potassium Phosphate (pH 6.5) 70.5 mM NADH 0.2 mM NAD 0.3 mM Lipoate 10.2 mM EDTA 0.81 mM BSA 0.7mg/mL
Wavelength for Measuremen (nm) Extinction coefficient (cm^2/μmol)	565 20	340 6.22

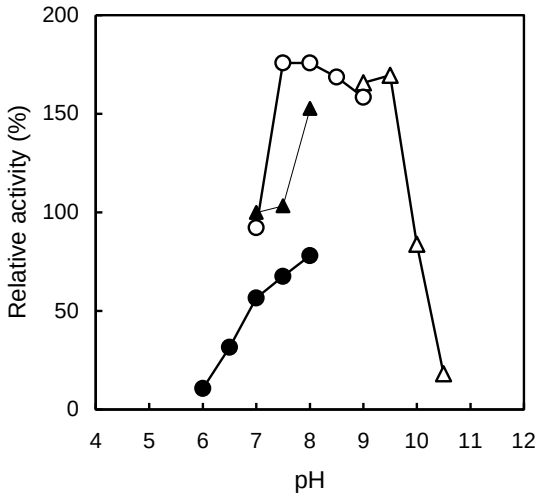

Fig. 1 pH profile

△ Gly-KOH, ○ Bicine,
● phosphate, ▲ HEPES

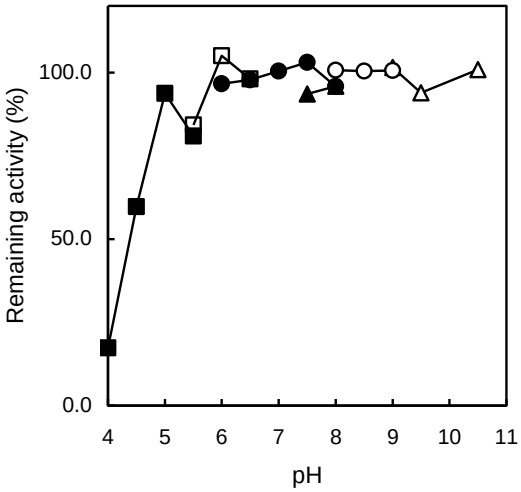

Fig. 2 pH stability

treated for 24 hr at 4℃ in the following buffer solution (0.1 M), :
△ Gly-KOH, ○Bicine,
● phosphate, ▲ HEPES,
□ MES, ■ Citrate

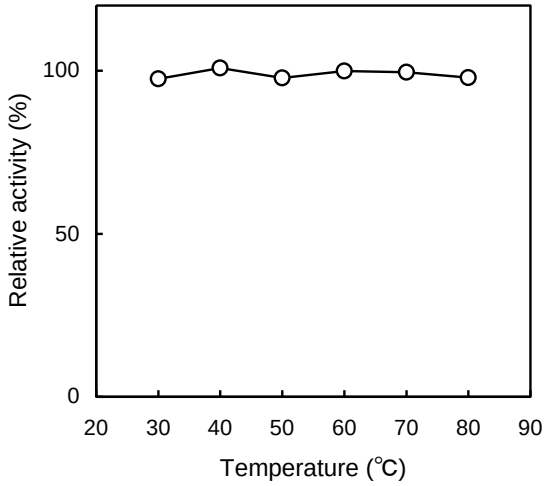

Fig. 3 Thermal stability

treated for 15 min in 0.1M potassium phosphate buffer, pH 7.5

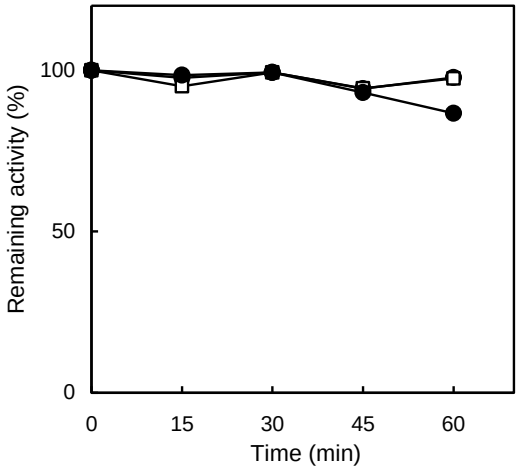

Fig. 4 Thermal stability

treated for in 0.1M potassium phosphate buffer, pH 7.5
○ 60℃, □ 70℃, ● 80℃

NIPRO ENZYMES

GALACTOSE DEHYDROGENASE (GalDH)

[EC 1. 1. 1. 48]

from recombinant *E. coli*

$$\text{D-Galactose} + \text{NAD(P)}^+ \leftrightarrow \text{D-Galactono-}\delta\text{-lactone} + \text{NAD(P)H} + \text{H}^+$$

SPECIFICATION

State	:	Ammonium sulphate suspension	
Specific activity	:	more than 80 U/mg protein	
Contaminants	:	(as GalDH activity = 100 %)	
		NADH oxidase	< 0.10 %
		LDH	< 0.10 %
		ADH	< 0.01 %

PROPERTIES

Subunit molecular weight	:	ca. 33,800	
Optimum pH	:	10.5	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Thermal stability	:	No significant decrease in activity up to 50 °C with Ammonium sulphate and 40 °C without Ammonium sulphate .(Fig. 3, 4)	
Michaelis constants	:	D-Galactose	0.25 mM
		NAD^+	0.15 mM
Substrate specificity (100mM)	:	D-Galactose	100 %
		D-Glucose	0.2 %
		D-Xylose	8.7 %
		D-Maltose	0.1 %
		D-Sucrose	0.1 %

STORAGE

Store at 4 to 10 °C (Do not freeze)
Stable at 4 °C for at least one year

APPLICATION

This enzyme is useful for determination of galactose.

ASSAY

Principle

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

$$\text{D-Galactose} + NAD^+ \xrightarrow{\text{GalDH}} \text{D-Galactono-}\delta\text{-lactone} + NADH + H^+$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH9.1 (at 30°C)
Ⅱ NAD^+ solution ; 100 mM
Ⅲ D-Galactose solution ; 1 M
Ⅳ Enzyme diluent ; 20 mM potassium phosphate, 0.1% bovine serum albumin, pH7.5

Preparation of Enzyme Solution

Dilute the enzyme suspension to approx. 5 U/mL with the enzyme diluent.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 27.60mL
   Solution Ⅱ 0.90mL
   Solution Ⅲ 1.50mL
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

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

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

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

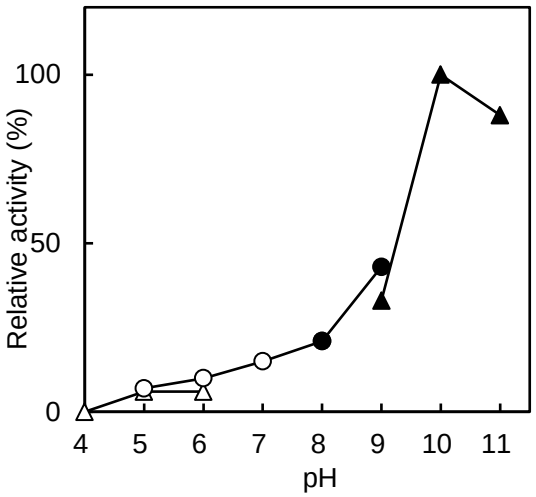

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ▲ Glycine-KOH

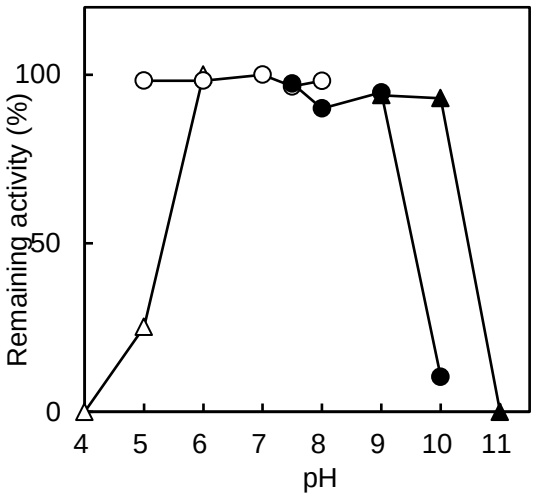

Fig. 2 pH stability

treated for 24 hr at 4 ℃ in the folowing buffer solution (0.1 M); △ acetate, ○ phosphate, ● Tris-HCl, ▲ Glycine-KOH

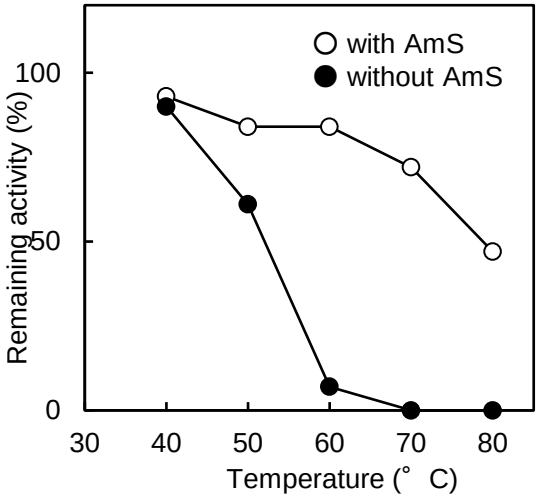

Fig. 3 Thermal stability

treated for 15 min in 25 mM potassium phosphate buffer pH 7.5, with or without 3.2 M ammonium sulphate (AmS).

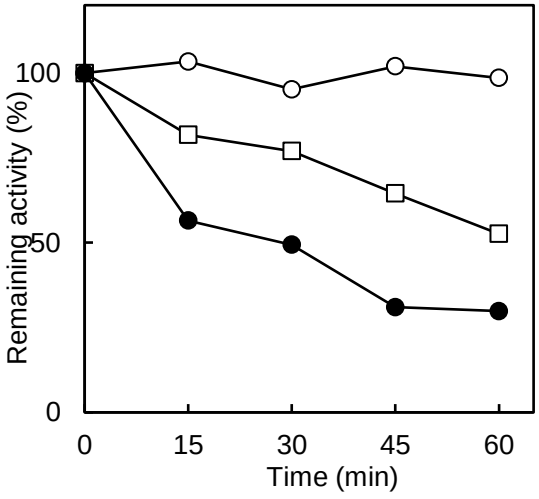

Fig. 4 Thermal stability

treated in 25 mM potassium phosphate buffer pH 7.5 at ○ 40 ℃, □ 50 ℃, ● 60 ℃ without ammonium sulphate.

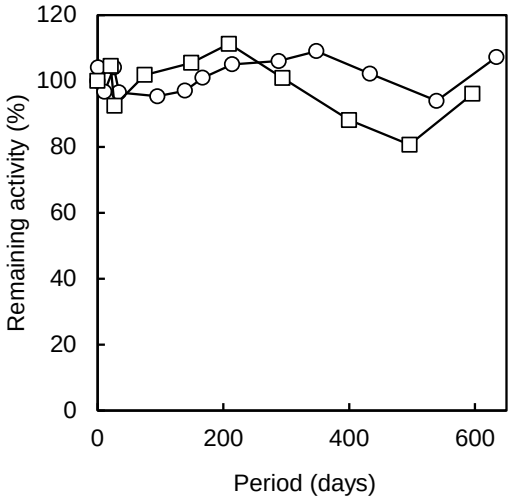

Fig. 5 Storage Stability

(ammonium suiphate suspension (ca. 1300U/mL) store at 4 ℃(○) or 10 ℃ (□).)

NIPRO ENZYMES

GLUCOKINASE 2 (GlcK2)

[EC 2. 7. 1. 2]

from *Recombinant E.coli*

ATP + D-Glucose ↔ ADP + D-Glucose 6-phosphate

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 350 U/mg protein	
Contaminants	:	(as GlcK2 activity = 100 %)	
		Glucose-6-phosphate dehydrogenase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		6-Phosphogluconate dehydrogenase	< 0.01 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Subunit molecular weight	:	ca. 32,000	
Optimum pH	:	9.0	(Fig. 1)
pH stability	:	7.0 - 10.0	(Fig. 2)
Optimum temperature	:	70 °C	(Fig. 5)
Thermal stability	:	No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	:	(60mM Tris-HCI buffer, pH 8.5, at 30 °C)	
		Glucose	0.1 mM
		ATP	0.05 mM
Substrate specificity	:	D-Glucose	100 %
		D-Mannose	20 %
		D-Fructose	0 %

STORAGE

Stable at -20°C for at least one year

APPLICATION

The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.

ASSAY

Principle

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

$$\text{ATP + Glucose} \xrightarrow{\text{GlcK2}} \text{ADP + Glucose 6-phosphate}$$

$$\text{Glucose 6-phosphate + NADP}^+ \xrightarrow{\text{G6PD}} \text{Gluconolactone 6-phosphate + NADPH + H}^+$$

Unit Definition

One unit of activity is defined as the amount of GlcK2 that forms 1 μmol of glucose 6-phosphate per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH 9.0
Ⅱ ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
Ⅲ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
Ⅳ $NADP^+$ solution ; 22.5 mM [(0.172 g NADP+ monosodium salt or 0.177 g NADP+ disodium salt)/10 mL distilled water]
Ⅴ Glucose solution ; 40 mM (0.072 g glucose (anhyd.)/10 mL distilled water)
Ⅵ Glucose-6-phosphate dehydrogenase (G6PDH) ; (from yeast. Roche Diagnostics K.K., No. 127 671) suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/2 mL) approx. 140 U/mg 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	17.97mL	SolutionⅣ	1.20mL
Solution Ⅱ	1.20 mL	Solution Ⅴ	9.00mL
SolutionⅢ	0.60 mL	SolutionⅥ	0.03mL

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

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

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

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

REFERENCE

1. Hengartner, H., and Zuber, H.; FEBS *Lett.*, **37**, 212 (1973)
2. Kamei, S., Tomita, K., Nagata, K., Okuno, H., Shiraishi, T., Motoyama, A., Ohkubo, A., and Yamanaka, H.; *J. Clin. Biochem. Nutr.*, **3**,1 (1987)
3. Tomita, K., Kamei, S., Nagata, K., Okuno, H., Shiraishi, T., Motoyama, A., Ohkubo, A., and

Yamanaka, M.; *ibid*., **3**, 11 (1987)

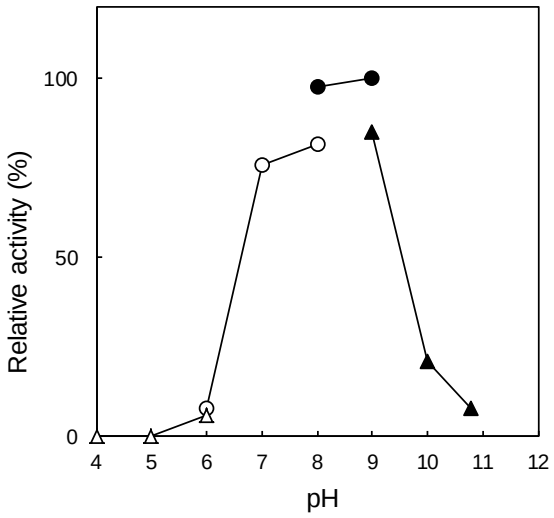

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ▲ carbonate

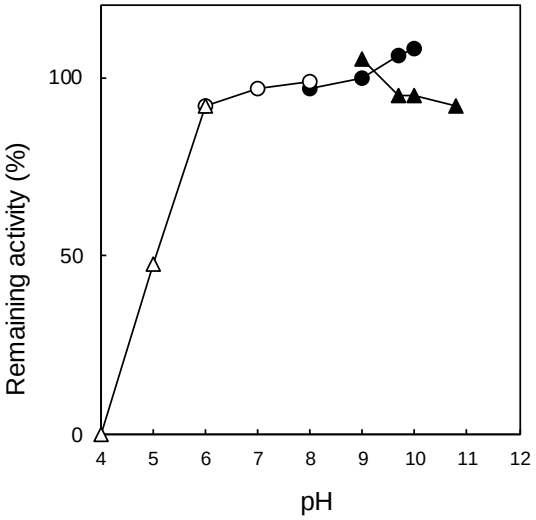

Fig. 2 pH stability

treated for 24 hr at 4℃ in the following buffer solution (0.1 M), :
△ acetate, ○ phosphate,
● Tris-HCl, ▲ carbonate

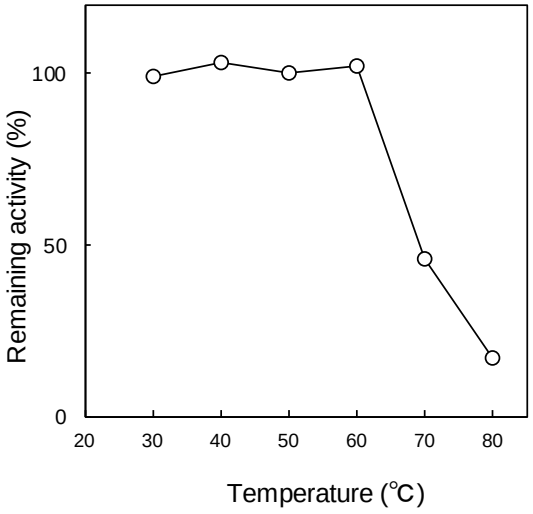

Fig. 3 Thermal stability

treated for 15 min. in 0.1 M Tris-HCl buffer, pH 8.9

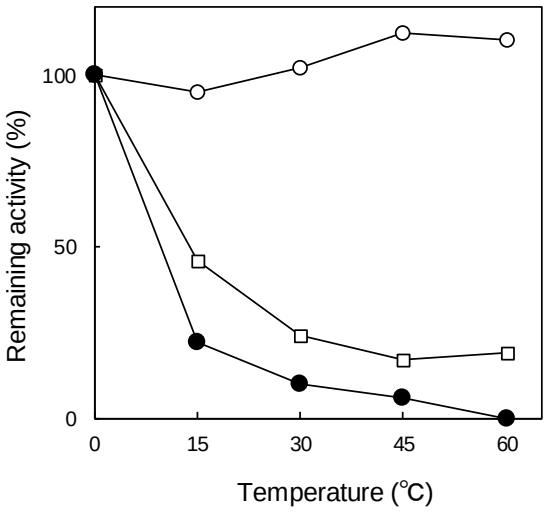

Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer , pH 8.9
○ 60℃, □70℃, ●80℃

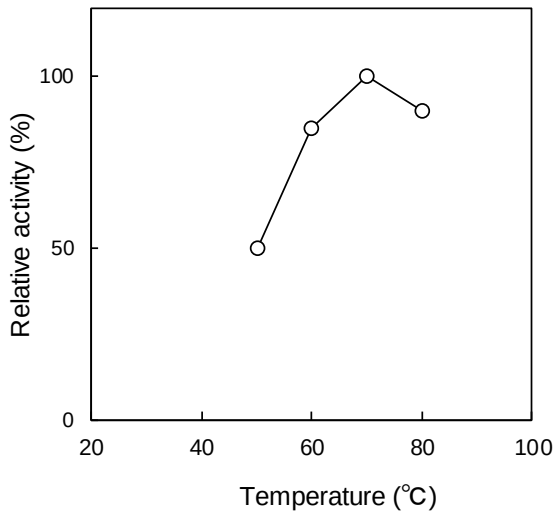

Fig. 5 Thermal activity

(defined as 100% at 70 °C)

NIPRO ENZYMES

GLUCOSE DEHYDROGENASE (GlcDH2)

[EC 1. 1. 1. 47]

from recombinant *E. coli*

$$D\text{-Glucose} + NAD(P)^+ \leftrightarrow D\text{-Glucono-}\delta\text{-lactone} + NAD(P)H + H^+$$

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 900 U/mg protein	
Contaminants	:	(as GlcDH2 activity = 100 %)	
		NADH oxidase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 126,000	
Subunit molecular weight	:	ca. 31,500	
Optimum pH	:	8.5	(Fig. 1)
pH stability	:	5.0 - 10.0 (with 3M NaCl)	(Fig. 2)
Thermal stability	:	No significant decrease in activity up to 70 °C.	
		(with 3M NaCl and 0.1% BSA)	(Fig. 3, 4)
Michaelis constants	:	D-Glucose	3.7 mM
		NAD^+	0.06 mM
		$NADP^+$	0.02 mM
Substrate specificity (100mM)	:	D-Glucose	100 %
		D-Maltose	1.1 %
		D-Galactose	0.1 %
		D-Xylose	3.0 %
		D-Fructose	0.3 %
		D-Mannose	4.8 %
		D-Arabinose	0 %
		Trehalose	0 %
		D-Lactose	1.3 %
		D-Sucrose	0 %
		2-Deoxy-D-Glucose	100 %
		D-Glucose-1-Phosphate	0 %
		D-Glucose-6-Phosphate	0 %
		D-Sorbitol	0 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

This enzyme is useful for determination of glucose.

ASSAY

Principle

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

$$\text{D-Glucose} + NAD^+ \xrightarrow{\text{GlcDH2}} \text{D-Glucono-}\delta\text{-lactone} + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of GlcDH2 that forms 1 μmol of NADH per minute at 37 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl, pH8.5 (at 25°C)
Ⅱ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)
Ⅲ D-Glucose solution ; 1 M (1.802 g glucose (anhyd.)/10 mL distilled water)
Ⅳ NaCl solution ; 5 M (2.92 g NaCl/10 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 15 U/mL with 20 mM potassium phosphate buffer containing 1mg/mL BSA and 2 M NaCl, pH 6.5.

Procedure

1. Prepare the following reaction mixture and pipette 2.70 mL of reaction mixture into a cuvette.
   Solution Ⅰ 17.22mL
   Solution Ⅱ 0.50mL
   Solution Ⅲ 2.00mL
   Solution Ⅳ 0.28mL
2. Incubate at 37 °C for about 3 minutes.
3. Add 0.015 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

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs_{340}) \times (2.70 + 0.015)}{6.22 \times 0.015} \times \text{d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH (cm^2/μmol)
*Protein concentration ; determined by the absorbance at 280nm (Abs280), where 1 Abs280 = 1 mg/mL

REFERENCE

1. Ramaley, R.F. and Vasantha, N.; *J. Biol. Chem.* **258**, 12558-12565 (1983)

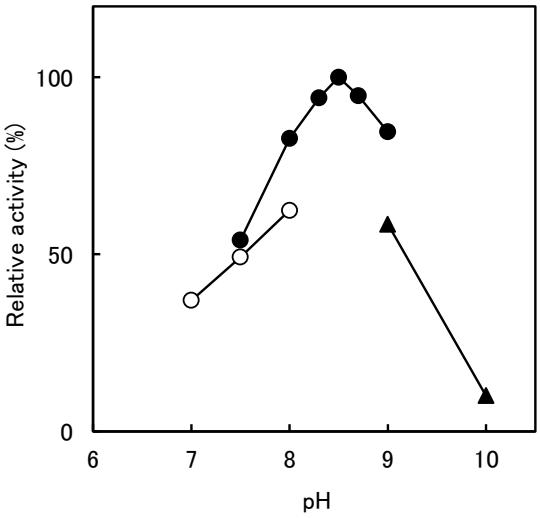

Fig. 1 pH profile

〔○ phosphate, ● Tris-HCl, ▲ glycine〕

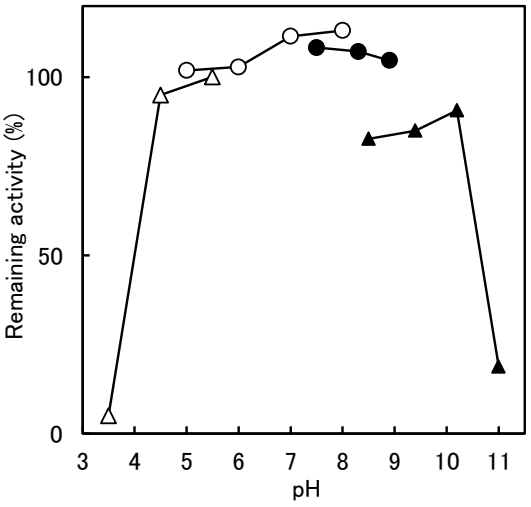

Fig. 2 pH stability

〔treated for 24 hr at 4℃ in the following buffer solution (0.1 M) containg 3M NaCl : △ acetate, ○ phosphate, ● Tris-HCl, ▲ glycine〕

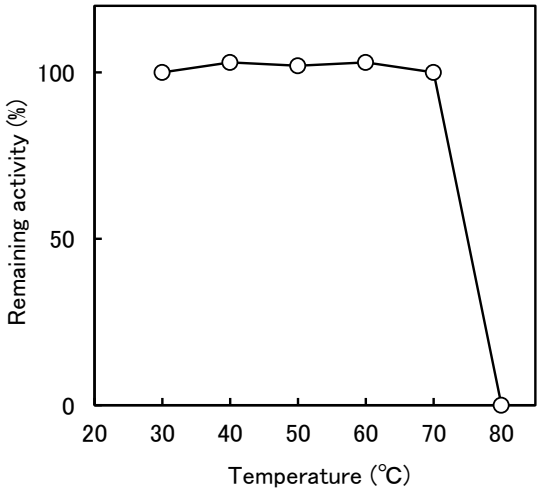

Fig. 3 Thermal stability

〔treated for 15 min in 0.1M phosphate buffer, pH 6.5, containing 3M NaCl and 0.1% BSA〕

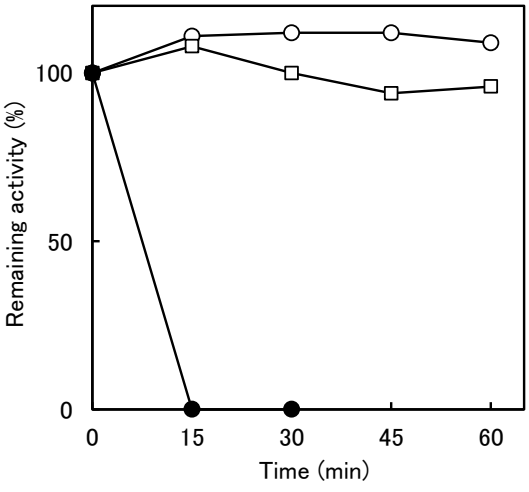

Fig. 4 Thermal stability

〔treated for in 0.1M phosphate buffer, pH 6.5, containing 3M NaCl and 0.1% BSA, ○ 60℃, □ 70℃, ● 80℃〕

NIPRO ENZYMES

D-LACTATE DEHYDROGENASE (D-LDH)

[EC 1. 1. 1. 28]

from *Microorganism*

$$D\text{-Lactate} + NAD^+ \leftrightarrow Pyruvate + NADH + H^+$$

FOR PYRUVATE → LACTATE REACTION

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 2,500 U/mg protein	
Contaminants	:	(as D-LDH activity = 100 %)	
		NADH oxidase	< 0.01 %
		GOT	< 0.01 %
		GPT	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 80,000	
Subunit molecular weight	:	ca. 40,000	
Optimum pH	:	7.5	(Fig. 1)
pH stability	:	5.5 - 10.0	(Fig. 2)
Isoelectric point	:	4.1	
Thermal stability	:	No detectable decrease in activity up to 40 °C.	(Fig. 3, 4)
Michaelis constants	:	(94 mM Potassium phosphate buffer, pH 7.5, at 30 °C)	
		Pyruvate	0.80 mM
		NADH	0.18 mM
Stabilizers	:	$(NH_4)_2 SO_4$, BSA	
Inhibitors	:	Zn_2^+, Cu_2^+	

STORAGE

Stable at -20 °C at least one year

ASSAY

Principle

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

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

Unit Definition

One unit is defined as the amount of D-LDH that forms 1 μmol of NAD^+ per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Potassium phosphate buffer, pH 7.5
Ⅱ Sodium pyruvate solution ; 100 mM (100 mg sodium pyruvate/10 mL distilled water)
Ⅲ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 3 to 5 U/mL with 50 mM potassium phosphate buffer containing 1 mg/mL BSA, pH 7.0.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 28.00mL
   Solution Ⅱ 1.20mL
   Solution Ⅲ 0.80mL
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

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

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

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

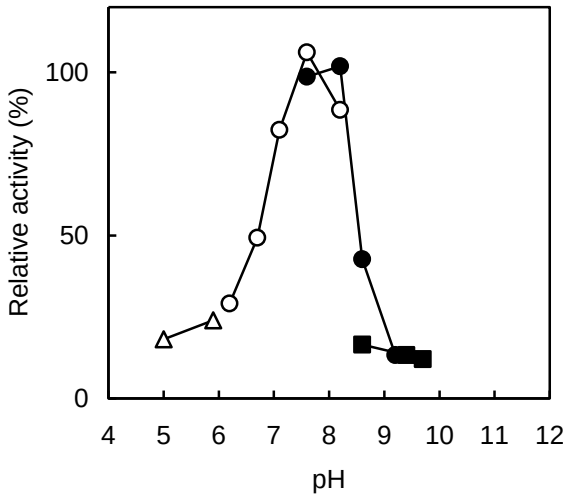

Fig. 1 pH profile

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

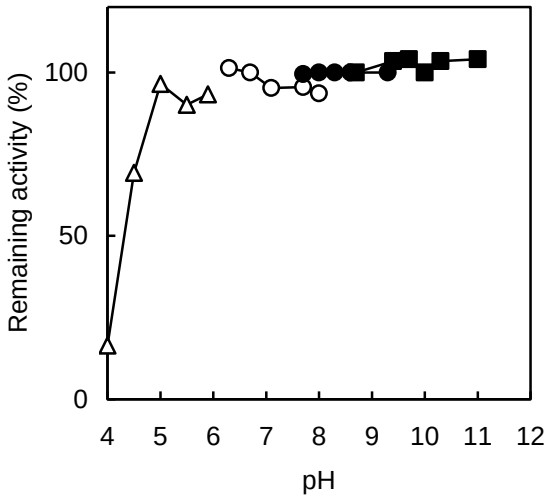

Fig. 2 pH stability

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

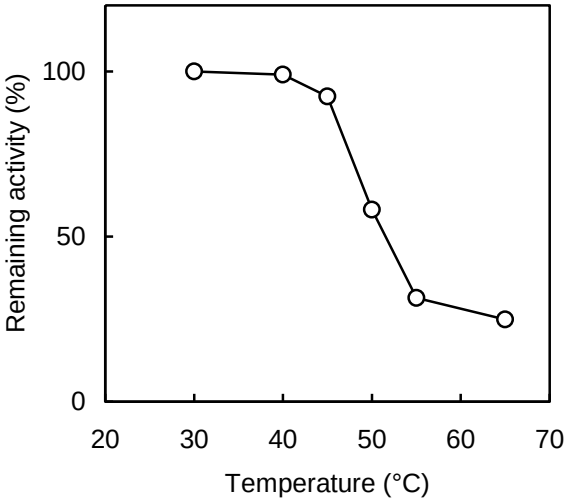

Fig. 3 Thermal stability

treated for 15 min in 0.1M potassium phosphate buffer, pH 7.0

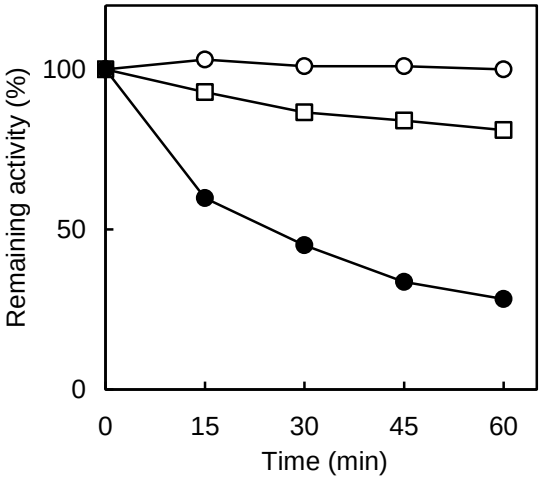

Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.0
○ 40 °C, □ 45 °C, ● 50 °C

NIPRO ENZYMES

MALATE DEHYDROGENASE (MDH)

[EC 1. 1. 1. 37]

from *Microorganism*

L-Malate+ NAD^+ ↔ Oxaloacetate + NADH + H^+

FOR OXALATE → MALATE REACTION

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 1,200 U/mg protein	
Contaminants	:	(as MDH activity = 100 %)	
		GOT	< 0.01 %
		GPT	< 0.01 %
		NADHoxidase	< 0.01 %
		Glutamate dehydrogenase	< 0.01 %
		Fumarase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 72,000	
Subunit molecular weight	:	ca. 36,000	
Optimum pH	:	9.0	(Fig. 1)
pH stability	:	5.5 - 11.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	:	(90mM Tris-HCI buffer, pH 9.0, at 30 °C)	
		Oxaloacetate	0.027 mM
		NADH	0.014 mM

STORAGE

Stable at -20 °C for at least six months

APPLICATION

This enzyme is useful for enzymatic determination of L- malate and of glutamate oxaloacetate transaminase in clinical analysis.

ASSAY

Principle

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

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^+$$

Unit Definition

One unit of activity is defined as the amount of MDH that forms 1 μmol of NAD^+ per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 200 mM Tris-HCl, pH 9.0
Ⅱ Oxaloacetate solution ; 15 mM (0.020 g oxaloacetate/10 mL distilled water)
Ⅲ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 3 to 5 U/mL with 100 mM Tris-HCl buffer, pH 9.0.

Procedure

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

Solution Ⅰ	13.50mL
Solution Ⅱ	1.00mL
Solution Ⅲ	0.57mL
H_2O	14.93mL

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

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

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

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

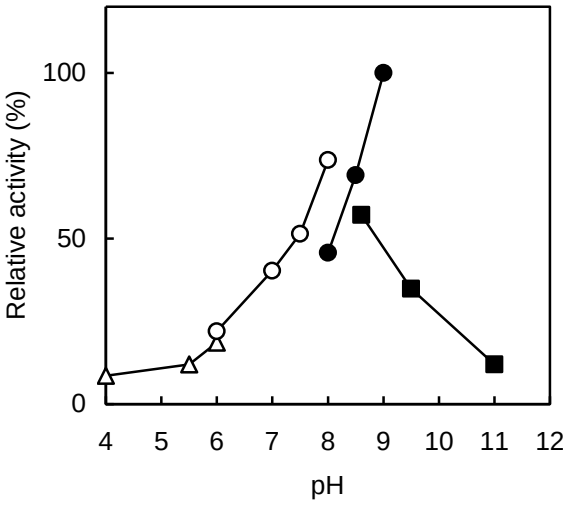

Fig. 1 pH profile

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

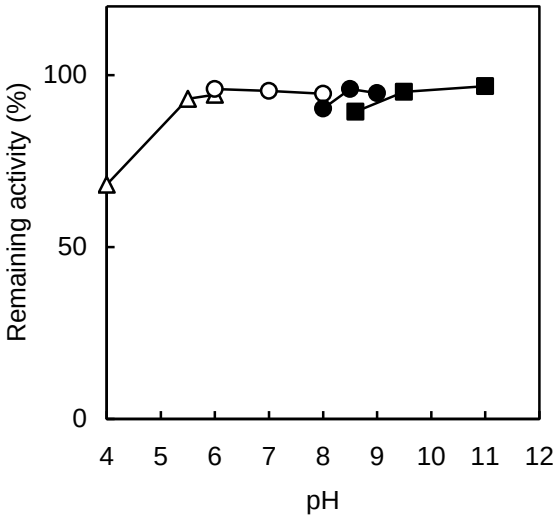

Fig. 2 pH stability

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

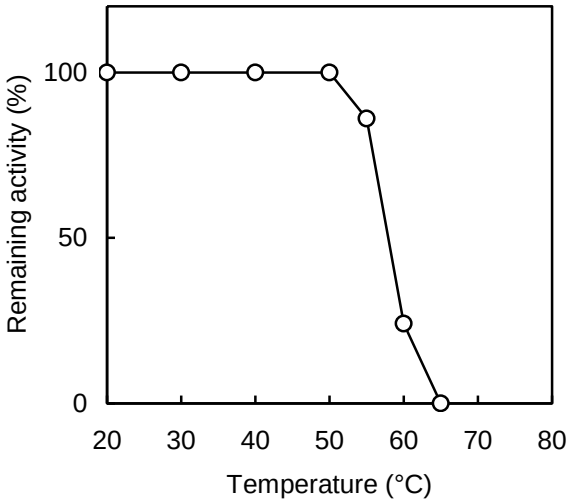

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tris-HCl buffer, pH 9.0

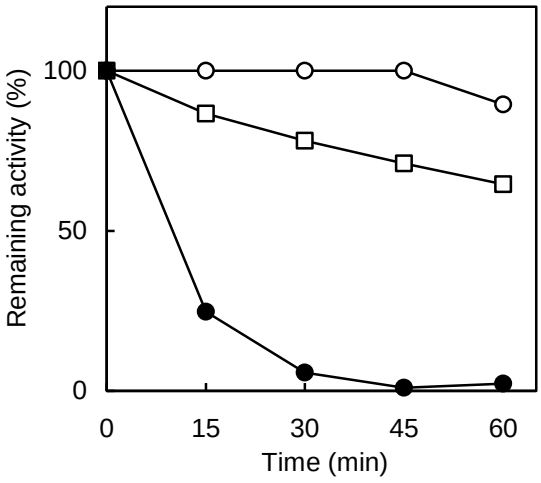

Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 9.0
○ 50 °C, □ 55 °C, ● 60 °C

NIPRO ENZYMES

MUTAROTASE (MRO)

[EC 5. 1. 3. 3]

from *Microorganism*

α-D-glucose ↔ β-D-glucose

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 120 U/mg protein	
Contaminants	:	(as MRO activity = 100 %)	
		NADHoxidase	< 0.01 %

PROPERTIES

Subunit molecular weight	:	ca. 39,500	
Optimum pH	:	7.0 - 9.0	(Fig. 1)
pH stability	:	3.5 - 10.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)

STORAGE

Stable at -20 °C for at least one year

APPLICATION

This enzyme is useful for enzymatic determination of glucose.

ASSAY

Principle

Acceleration of the glucose dehydrogenase reaction by Mutarotase is measured according to the following reactions.

$$\text{sucrose} \xrightarrow{\beta\text{-Fructosidase}} \alpha\text{-glucose} + \text{fructose}$$

$$\alpha\text{-glucose} \xrightarrow{\text{MRO}} \beta\text{-glucose}$$

$$\beta\text{-glucose} + NAD^+ \xrightarrow{\text{GlucoseDH}} \text{glucono-lactone} + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of Mutarotase that forms 10μmol of NADH per minute at 25 °C.

Solutions

Ⅰ HEPES buffer ; 50 mM (1.19 g HEPES / 100 mL distilled water, adjust pH to 7.5 with NaOH)
Ⅱ Sucrose solution ; 16.7 mM (57 mg Sucrose / 10 mL distilled water)
Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid / 10 mL distilled water)
Ⅳ Glucose dehydrogenase solution ; 3 kU/mL (GlcDH2, Nipro Corp. / 20 mM potassium phosphate containing 2M NaCl, pH6.5)
Ⅴ β-Fructosidase solution ; ≧30 kU/mL (100 mg Invertase from baker's yeast, Sigma-Aldrich I4504 / 1 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 0.7 to 1.4 U/mL with the enzyme dilutent (20 mM potassium phosphate pH7.3 containing 1mg/mL BSA).

Procedure

1. Prepare the following reaction mixture and pipette 2.70 mL of reaction mixture into a cuvette.

Solution Ⅰ	19.90mL	SolutionⅣ	0.166mL
Solution Ⅱ	1.00mL		
SolutionⅢ	0.60mL		

2. Add 0.015 mL of the enzyme solution into the cuvette and mix.
3. Incubate at 25 °C for about 3 minutes.
4. Add 0.06 mL of the Solution Ⅴ into the cuvette and mix.
5. Read absorbance change at 340nm per minute (ΔAbs1) in the linear portion of curve.
6. Run the procedure 1 to 5 with the enzyme diluent instead of the enzyme solution (ΔAbs2).

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta Abs1 - \Delta Abs2) \times (2.70 + 0.015 + 0.060)}{6.22 \times 0.015 \times 10} \times \text{d.f.}$$

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

d.f. ; dilution factor
6.22 ; millimolar extinction coefficient of NADH ($cm^2/\mu mol$)
10 ; conversion factor
*Protein concentration ; determined by the absorbance at 280nm (Abs280), where 1 Abs280 = 1 mg/mL

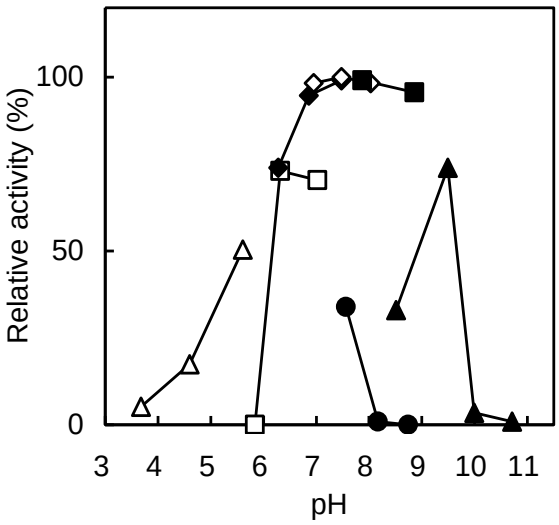

Fig. 1 pH profile

△ acetate, □ MES, ◆ PIPES,
◇ HEPES, ● Tris-HCl, ■ Bicine,
▲ Glycine-KOH

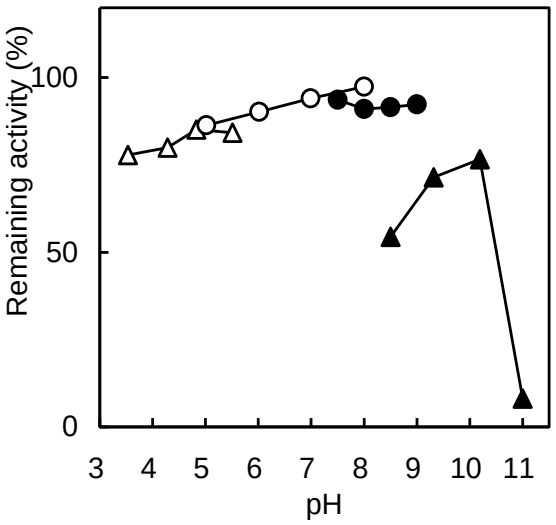

Fig. 2 pH stability

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

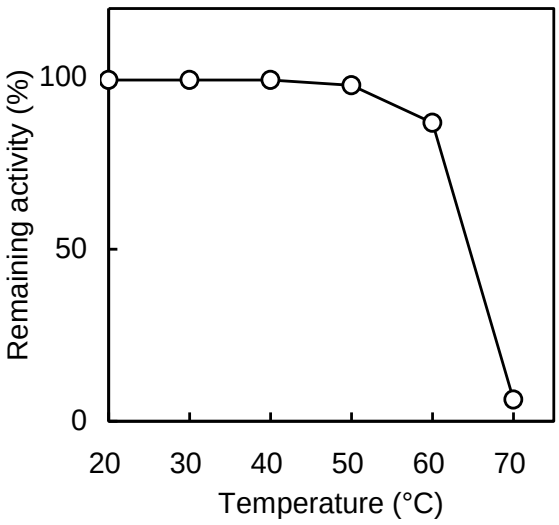

Fig. 3 Thermal stability

treated for 15 min in 0.1 M potassium phosphate buffer pH 6.5, 0.1 % BSA.

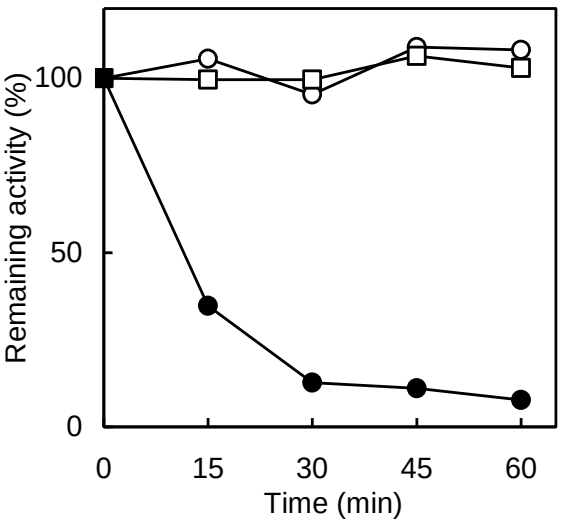

Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer pH 6.5, 0.1 % BSA at ○ 40 °C, □ 50 °C, ● 60 °C.

NIPRO ENZYMES

PHENYLALANINE DEHYDROGENASE (PheDH)

[EC 1.4.1.20]

from *Thermoactinomyces intermedius*

L-Phenylalanine + NAD^+ + H_2O ↔ Phenylpyruvate + NH_4^+ + NADH

SPECIFICATION

State	:	Ammonium sulphate suspension	
Specific activity	:	more than 30 U/mg protein	
Contaminants	:	(as PheDH activity = 100 %)	
		NADH oxidase	< 0.01 %
		Lactate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 380,000	
Subunit molecular weight	:	ca. 40,000	
Optimum pH	:	11.5	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	:	(200 mM Gly-KCl-KOH buffer, pH 11.0, at 30 °C)	
		L-Phenylalanine	0.66 mM
		NAD^+	0.05 mM
Substrate specificity	:	L-Phenylalanine	100 %
		L-Tyrosine	7.6 %
		L-Methionine	1.5 %

STORAGE

Stable at 0 to 4 °C for at least six months (Do not freeze)

ASSAY

Principle

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

$$\text{L-Phenylalanine} + NAD^+ + H_2O \xrightarrow{\text{PheDH}} \text{Phenylpyruvate} + NH_4^+ + NADH$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 400 mM Gly-KCl-KOH, pH 11.0
Ⅱ L-Phenylalanine solution ; 100 mM (0.165 g L-phenylalanine/10 mL distilled water)
Ⅲ NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)

Preparation of Enzyme Solution

Dilute the ammonium sulphate suspension of enzyme to 2 to 6 U/mL with 10 mM Tris-HCl buffer, pH 8.0, containing 50 mM NaCl.

Procedure

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

Solution Ⅰ	15.00mL
Solution Ⅱ	3.00mL
Solution Ⅲ	0.15mL
H_2O	11.85mL

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 340nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

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

REFERENCE

1. Ohshima, T., Takada, H., Yoshimura, T., Esaki, N., and Soda, K.; *J. Bacteriol.*, **173**, 3943 (1991)

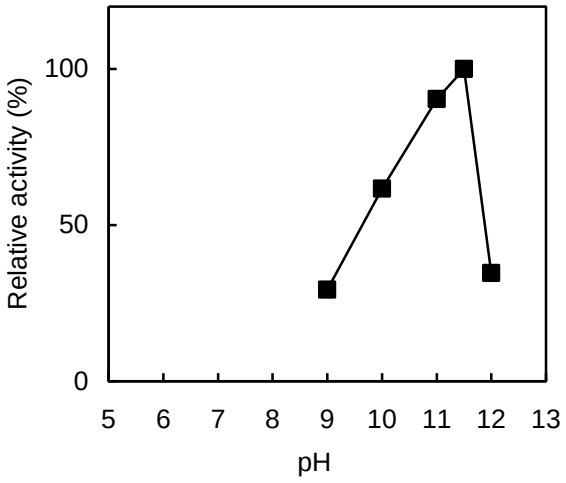

Fig. 1 pH profile

■ Gly-KCl-KOH

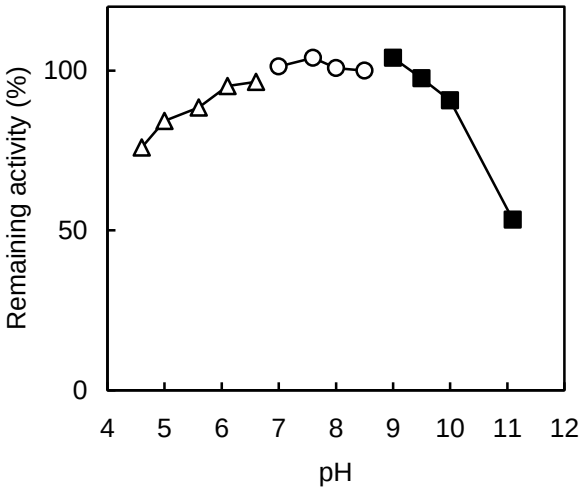

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (50 mM);
△ acetate, ○ phosphate,
■ Gly-KCl-KOH

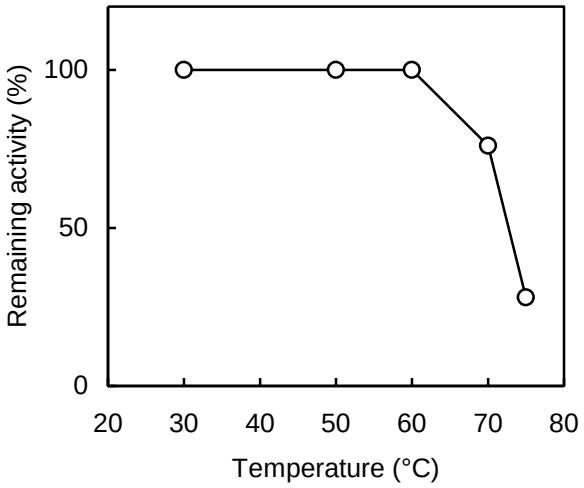

Fig. 3 Thermal stability

treated for 15 min in 10 mM potassium phosphate buffer, pH 7.2

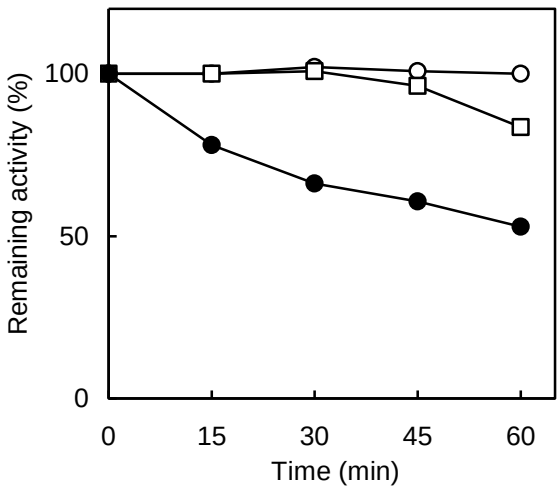

Fig. 4 Thermal stability

treated in 10 mM potassium phosphate buffer, pH 7.2
○ 50 °C, □ 60 °C, ● 70 °

NIPRO ENZYMES

6-PHOSPHOGLUCONATE DEHYDROGENASE (DECARBOXYLATING) (6PGDH)

[EC 1. 1. 1. 44]

from *Microorganism*

6-Phospho-D-gluconate + NAD^+ ↔ D-Ribulose 5-phosphate + CO_2 + NADH + H^+

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 40 U/mg protein	
Contaminants	:	(as 6PGDH activity = 100 %)	
		Glucokinase	< 0.01 %
		Phosphoglucomutase	< 0.01 %
		Hexose-6-phosphate isomerase	< 0.01 %
		Glutathione reductase	< 0.01 %

PROPERTIES

Molecular weight	:	ca. 132,000	
Subunit molecular weight	:	ca. 33,000	
Optimum pH	:	7.0 - 7.5	(Fig. 1)
pH stability	:	5.0 - 10.0	(Fig. 2)
Isoelectric point	:	ca. 4.5	
Thermal stability	:	(50 mM MES-NaOH buffer, pH 6.8, containing 0.5 M KCl)	
		No detectable decrease in activity up to 40 °C.	(Fig. 3, 4)
Michaelis constants	:	(80 mM GIycylglycine buffer, pH 7.5, at 30 °C)	
		6-Phospho-D-gluconate	0.95 mM
		NAD^+	0.32 mM
Stabilizer	:	KCl, $MgCl_2$, Sorbitol, BSA	
Activators	:	Mg^{2+}, Mn^{2+}, Ca^{2+}, K^+, Na^+	
Inhibitors	:	Fructose 1,6-bisphosphate, Erythrose 4-phosphate, NADH	

STORAGE

Stable at -20 °C for at least six months

ASSAY

Principle

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

$$\text{6-Phosphogluconate} + NAD^+ \xrightarrow{\text{6PGDH}} \text{Ribulose 5-phosphate} + CO_2 + NADH + H^+$$

Unit Definition

One unit of activity is defined as the amount of 6PGDH that forms 1 μmol of NADH per minute at 30 °C.

Solutions

Ⅰ Buffer solution ; 100 mM Glycylglycine-NaOH, pH 7.5
Ⅱ 6-Phospho-D-gluconate (6PG) solution ; 100 mM (0.378g 6PG trisodium salt·$2H_2O$/10 mL distilled water)
Ⅲ NAD^+ solution ; 50 mM (0.332 g NAD^+ free acid/10 mL distilled water)
Ⅳ $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 100 mM MES-NaOH buffer containing 1 mg/mL BSA, pH 6.8.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   Solution Ⅰ 24.6mL
   Solution Ⅱ 3.0mL
   Solution Ⅲ 2.1mL
   Solution Ⅳ 0.3mL
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

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

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

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

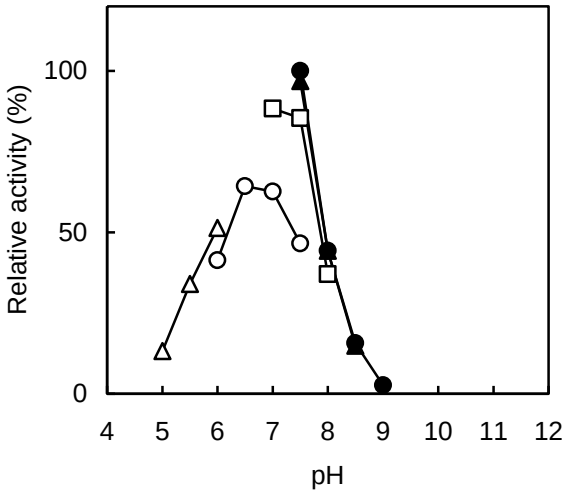

Fig. 1 pH profile

△ acetate, ○ phosphate,
□ TEA-NaOH, ▲ GlyGly-NaOH,
● Tris-HCl

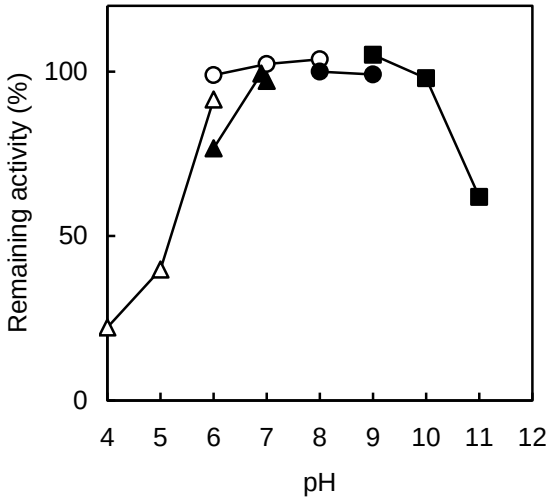

Fig. 2 pH stability

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

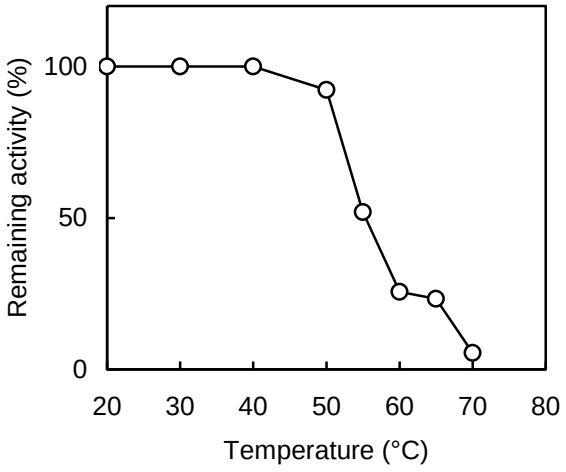

Fig. 3 Thermal stability

treated for 15 min in 50 mM MES-NaOH buffer, pH 6.8, containing 0.5 M KCl

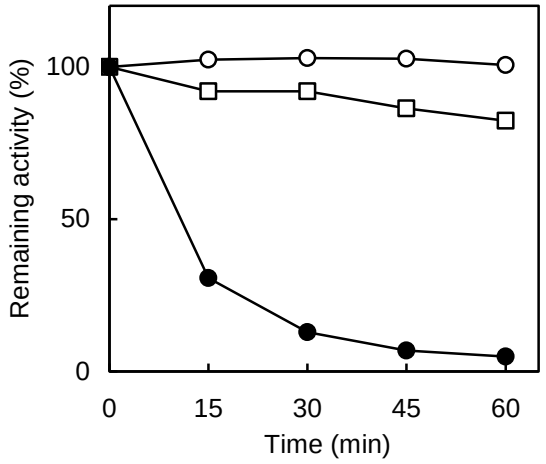

Fig. 4 Thermal stability

treated in 50 mM MES-NaOH buffer, pH 6.8, containing 0.5 M KCl
○ 40 °C, □ 50 °C, ● 60 °C

NIPRO ENZYMES

SORBITOL DEHYDROGENASE (SorDH)

[EC 1.1.1.14]

from *Microorganism*

D-Sorbitol + NAD^+ ↔ D-Fructose + NADH + H^+

SPECIFICATION

State	:	Lyophilized	
Specific activity	:	more than 30 U/mg protein	
Contaminants	:	(as SorDH activity = 100 %)	
		NADH oxidase	<0.01 %

PROPERTIES

Molecular weight	:	ca. 68,000	
Subunit molecular weight	:	ca. 26,000	
Optimum pH	:	11.0	(Fig. 1)
pH stability	:	6.0 - 10.0	(Fig. 2)
Optimum temperature	:	40 °C	
Thermal stability	:	No detectable decrease in activity up to 35 °C.	(Fig. 3, 4)
Michaelis constants	:	(100 mM Tris-HCl buffer, pH 9.0, at 30°C)	
		D-Sorbitol	3.4 mM
		NAD^+	0.13 mM
Substrate specificity	:	D-Sorbitol	100 %
		Galactitol	27 %
		L-Iditol	42 %
		Xylitol	1 %
		D-Arabitol	0 %
		D-Mannitol	0 %
		D-Glucose	0 %
		D-Galactose	0 %
		Maltose	0 %

STORAGE

Stable at -20 °C for at least one year

APPLICATION

This enzyme is useful for determination of D-Sorbitol in clinical analysis and food analysis.

ASSAY

Principle

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

$$\text{D-Sorbitol} + NAD^+ \xrightarrow{\text{SorDH}} \text{D-Fructose} + NADH + H^+$$

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 100 mM Tris-HCl buffer, pH 9.0
Ⅱ NAD^+ solution ; 20 mM (133 mg NAD^+ free acid /10 mL distilled water)
Ⅲ D-Sorbitol solution ; 500mM (911 mg D-Sorbitol/10 mL 100 mM Tris-HCl buffer, pH 9.0)

Preparation of Enzyme Solution

Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 10 U/mL with 50mM Tris-HCl buffer containing 1 mg/mL BSA, pH 8.0.

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   solution Ⅰ 24.00mL
   solution Ⅱ 3.00mL
   solution Ⅲ 3.00mL
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 the curve.

Calculation

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

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

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

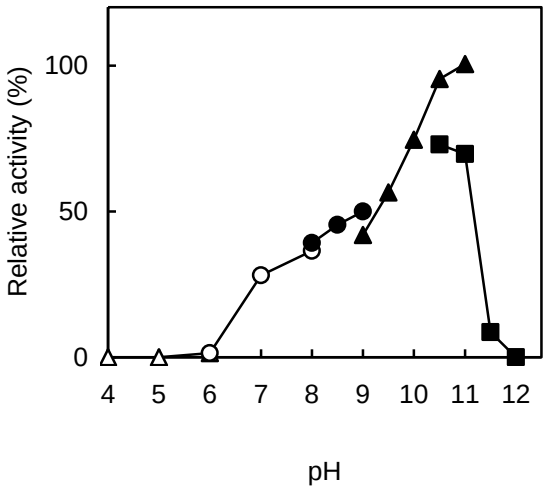

Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl, ▲Gly-KOH,
■ Na_2HPO_4-NaOH

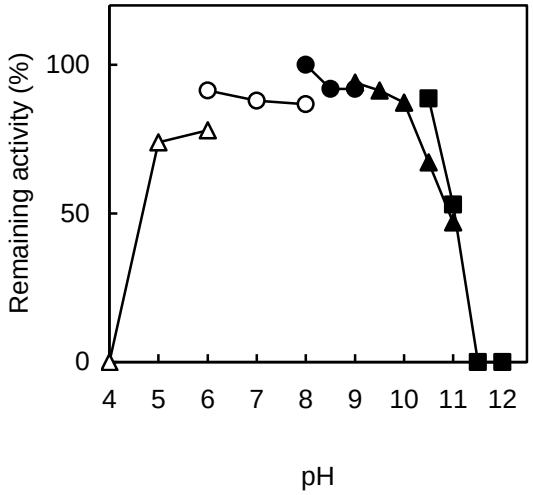

Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
● Tris-HCl, ▲ Gly-KOH,
■ Na_2HPO_4-NaOH

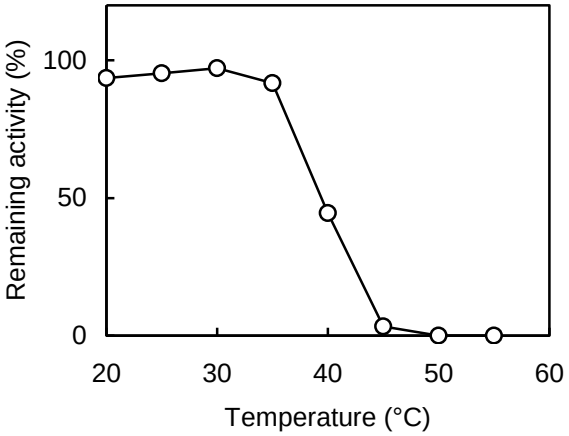

Fig. 3 Thermal stability

treated for 15 min in 0.1 M Tricine buffer, pH 8.0

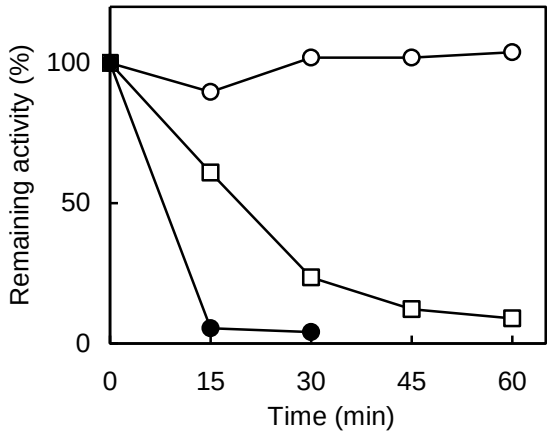

Fig. 4 Thermal stability

treated in 0.1 M Tricine -NaOH buffer, pH 8.0
○ 35 °C, □ 40 °C, ● 45 °C