

**Thermostable Enzymes for
Clinical Chemistry**

UNITIKA
ENZYMES

From *Zymomonas mobilis*

	PRODUCT CODE No.	
ALCOHOL DEHYDROGENASE (ZM-ADH)	Z 1 A 1 1 1	1
GLUCOKINASE (ZM-GlcK)	Z 2 G 1 1 1	4
GLUCOSE-6-PHOSPHATE DEHYDROGENASE (ZM-G6PDH)	Z 1 G 1 1 1	7

From *Bacillus stearothermophilus*

ACETATE KINASE (AK)	B 2 A 1 1 1	10
ADENYLATE KINASE (AdK)	B 2 A 2 1 1	14
ALANINE DEHYDROGENASE (AlaDH)	B 1 A 1 2 1	18
ALANINE RACEMASE (AlaR)	B 5 A 1 1 1	21
DIAPHORASE I [EC 1.6.99.-] (Di-1)	B 1 D 1 1 1	24
DIAPHORASE II [EC 1.8.1.4] (Di-2)	B 1 D 2 1 1	30
GLUCOKINASE (GlcK)	B 2 G 1 1 1	34
GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH)	B 1 G 1 1 1	38
α -GLUCOSIDASE (α -Glu)	B 3 G 1 1 1	42
GLUTAMINE SYNTHETASE (GS)	B 6 G 1 1 1	45
LEUCINE DEHYDROGENASE (LeuDH)	B 1 L 1 2 1	49
PHOSPHOFRUCTOKINASE (PFK)	B 2 P 1 1 1	52
PHOSPHOGLUCOSE ISOMERASE (PGI)	B 5 P 1 1 1	56
PHOSPHOGLYCERATE KINASE (PGK)	B 2 P 2 1 1	59
PHOSPHOTRANSACETYLASE (PTA)	B 2 P 3 1 1	62
POLYNUCLEOTIDE PHOSPHORYLASE (PNPase)	B 2 P 4 1 1	65
PYRUVATE KINASE (PK)	B 2 P 5 1 1	72
SUPEROXIDE DISMUTASE (SOD)	B 1 S 1 1 1	75

From Others

GALACTOSE DEHYDROGENASE (GalDH) *Available Soon	M 1 G 5 2 5	79
GLUCOSE DEHYDROGENASE (GlcDH2)	M 1 G 2 2 1	82
D-LACTATE DEHYDROGENASE (D-LDH)	M 1 L 1 1 1	85
MALATE DEHYDROGENASE (MDH)	M 1 M 1 1 1	88
MUTAROTASE (MRO)	M 5 M 1 2 1	91
PHENYLALANINE DEHYDROGENASE (PheDH)	M 1 P 1 2 5	94
6-PHOSPHOGLUCONATE DEHYDROGENASE (6PGDH)	M 1 P 2 1 1	97
SORBITOL DEHYDROGENASE (SorDH)	M 1 S 1 1 1	100

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

ALCOHOL DEHYDROGENASE (ZM-ADH)

[EC 1 .1 .1 .1]

from *Zymomonas mobilis*



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

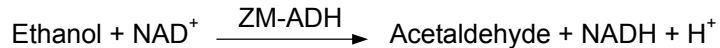
APPLICATION

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.



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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*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)

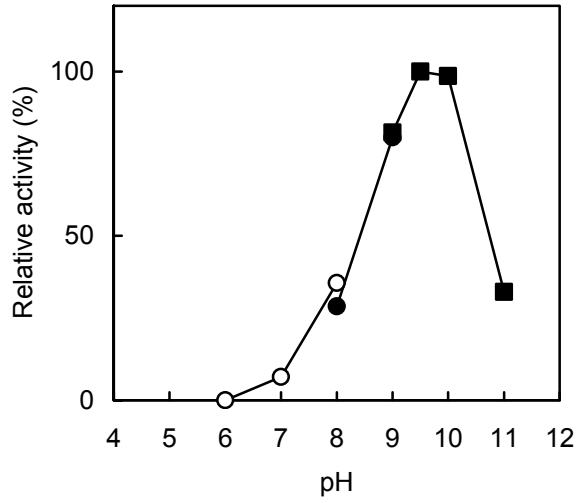


Fig. 1 pH profile

(○ phosphate, ● Tris-HCl, ■ Gly-KOH)

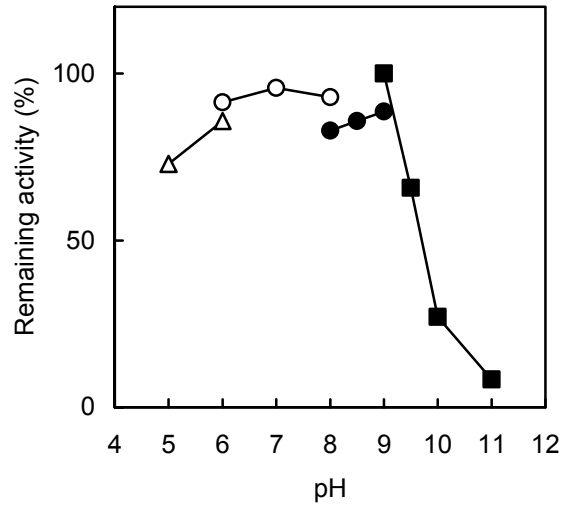


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the following buffer solution (0.1 M), containing 0.5 mM CoCl₂;

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

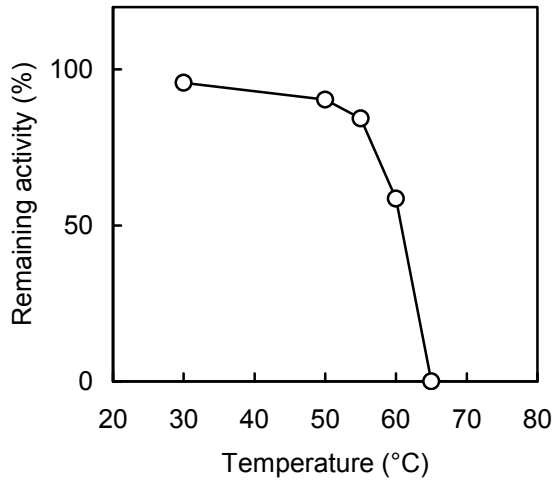


Fig. 3 Thermal stability

(treated for 15 min in 0.1M phosphate buffer containing 0.2 mM CoCl₂, pH 6.5)

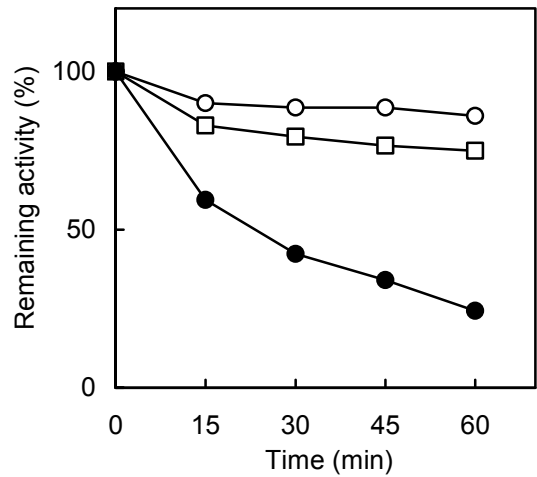


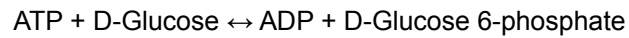
Fig. 4 Thermal stability

(treated in 0.1 M phosphate buffer containing 0.2 mM CoCl₂, pH 6.5
○ 50 °C, □ 55 °C, ● 60 °C)

GLUCOKINASE (ZM-GlcK)

[EC 2. 7. 1. 2]

from *Zymomonas mobilis*



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

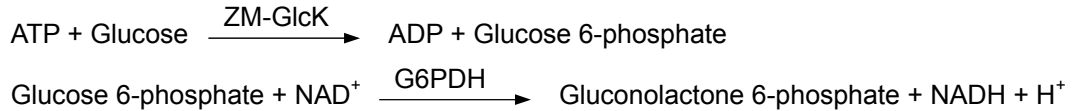
The enzyme is useful for diagnostic reagent, for example, glucose determination or CK determination, and for the specific determination of glucose.

Tris-HCl 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.



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- $3\text{H}_2\text{O}$ /(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
- III MgCl_2 solution ; 1 M (20.33 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ /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*, Unitika Ltd., 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
Solution III	0.30mL	Solution VI	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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*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)

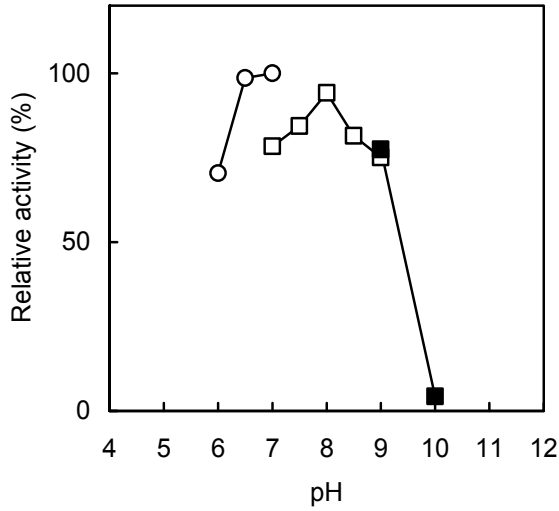


Fig. 1 pH profile

(○ MES-KOH, □ TEA-NaOH,
■ Gly-KOH)

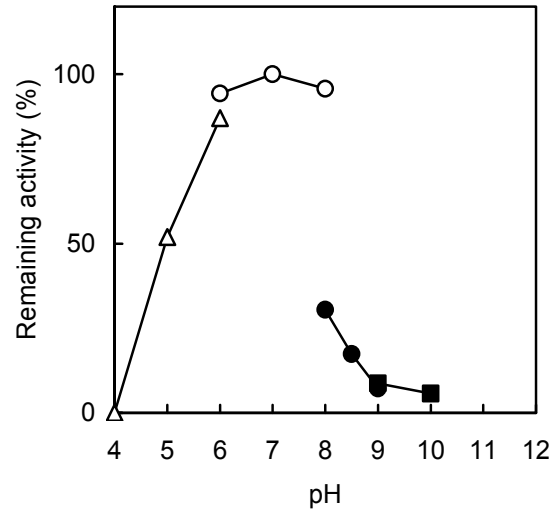


Fig. 2 pH stability

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

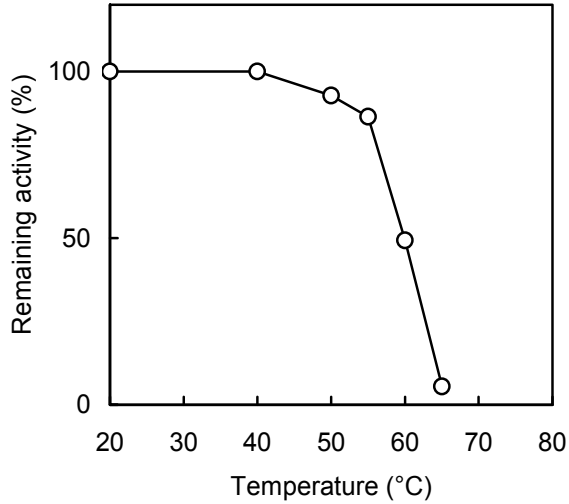


Fig. 3 Thermal stability

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

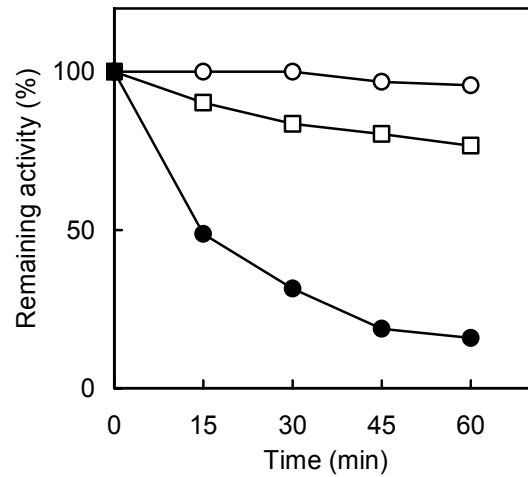


Fig. 4 Thermal stability

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

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (ZM-G6PDH)

[EC 1. 1. 1. 49]

from *Zymomonas mobilis*



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

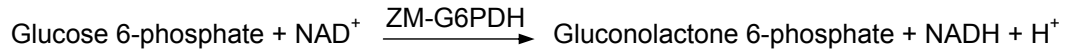
APPLICATION

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.



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 $2\text{H}_2\text{O}$ /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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*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)

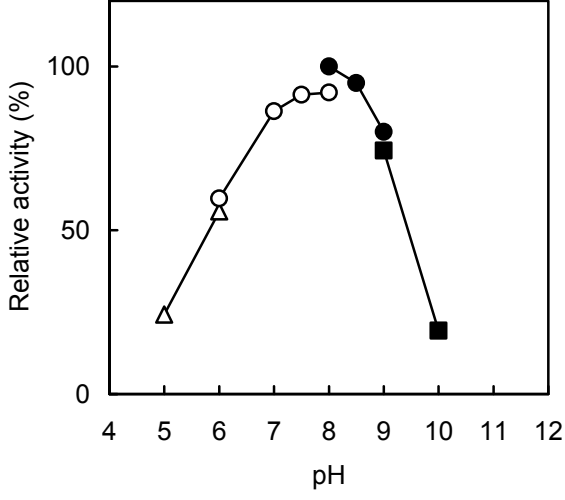


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacksquare Gly-KOH)

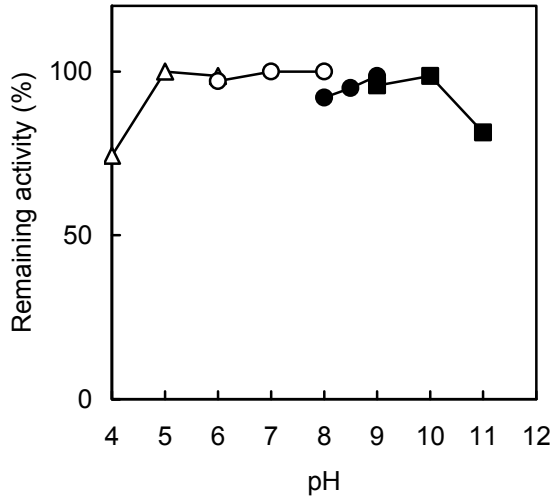


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
 Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacksquare Gly-KOH)

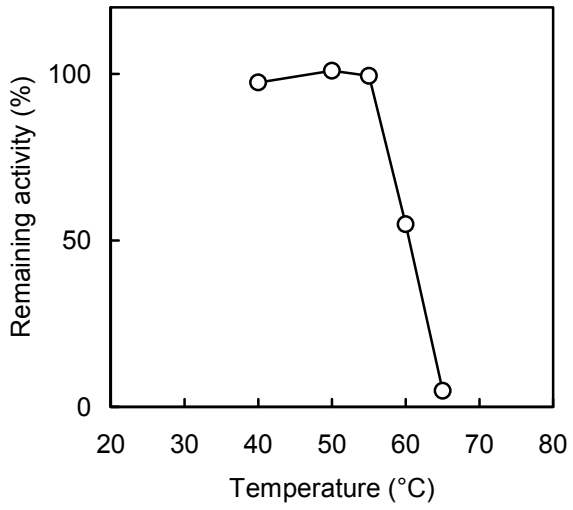


Fig. 3 Thermal stability

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

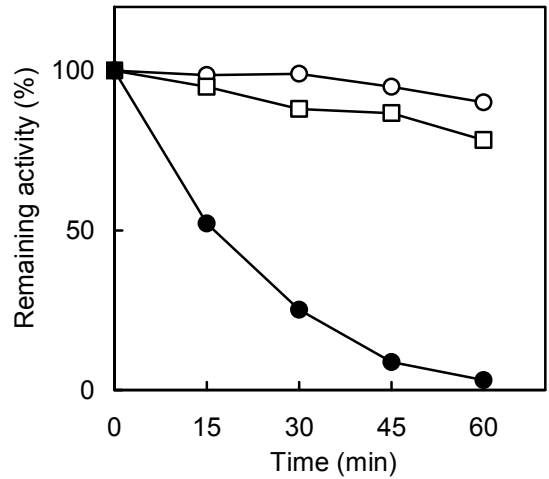


Fig. 4 Thermal stability

(treated in 0.1 M phosphate buffer, pH 7.0
 \circ 50 °C, \square 55 °C, \bullet 60 °C)

ACETATE KINASE (AK)

[EC 2. 7. 2. 1]

from *Bacillus stearothermophilus*



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- HCl buffer, pH 7.2, at 30 °C)	
	Acetate	120 mM
	Acetylphosphate	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

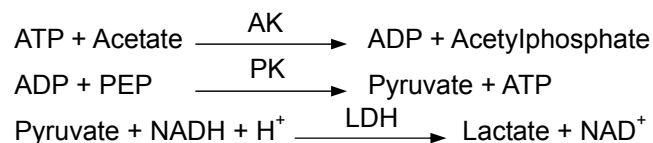
APPLICATION

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.



Unit Definition

One unit of activity is defined as the amount of AK that forms 1 μmol of ADP per minute at 30 $^{\circ}\text{C}$.

Solutions

- I Buffer solution ; 100 mM Imidazole-HCl, pH 7.2
- II ATP solution ; 100 mM (0.605 g ATP disodium salt-3H₂O/(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-3H₂O/10 mL distilled water)
- V MgCl₂ solution ; 1 M (20.33 g MgCl₂·6H₂O /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 (NH₄)₂SO₄ solution (10mg/mL) approx. 200 U/mg at 25 $^{\circ}\text{C}$
- VIII Lactate dehydrogenase (LDH) ; (from hog muscle, Roche Diagnostics K.K., No. 127 221) 50 % glycerol solution (25 mg/2.5 mL) approx. 550 U/mg at 25 $^{\circ}\text{C}$
- IX Sodium acetate solution ; 2 M (27.22g sodium acetate-3H₂O/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 $^{\circ}\text{C}$ 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 minutes (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*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)

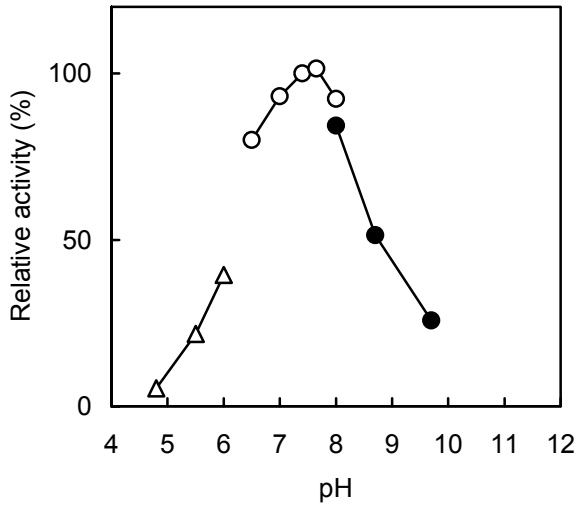


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
 \bullet Tris-HCl)

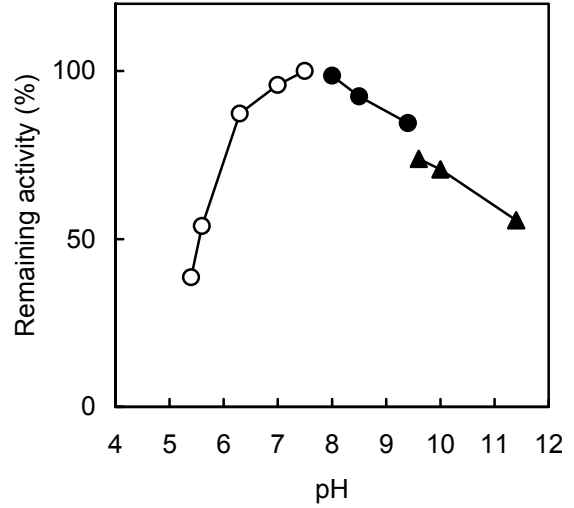


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
 \circ phosphate, \bullet Tris-HCl,
 \blacktriangle carbonate)

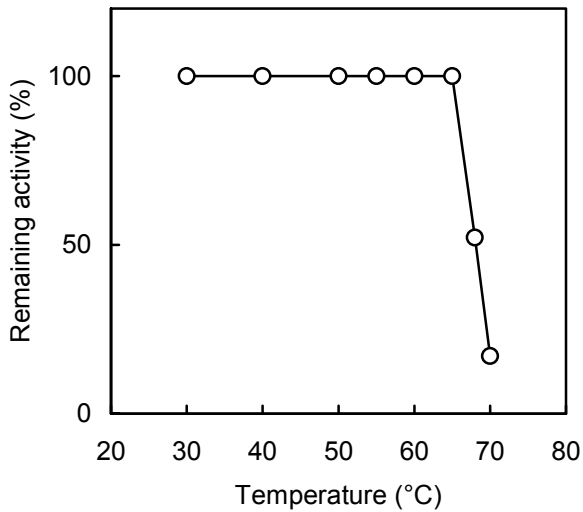


Fig. 3 Thermal stability

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

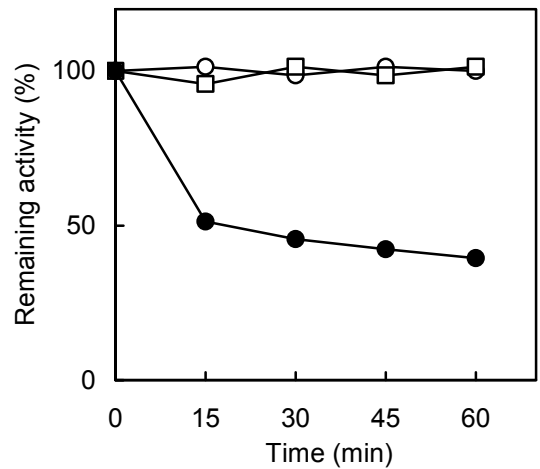


Fig. 4 Thermal stability

(treated in 0.1M potassium phosphate buffer, pH 7.5
 \circ 60 °C, \square 65 °C, \bullet 70 °C)

ADENYLATE KINASE (AdK)

[EC 2. 7. 4. 3]

from *Bacillus stearothermophilus*



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-HCl 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

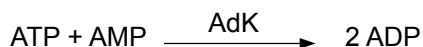
APPLICATION

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.



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-6H₂O/10 mL distilled water)
- III ATP solution ; 100 mM (0.605 g ATP disodium salt-3H₂O/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
- IV NADH solution ; 13.1 mM (0.100 g NADH disodium salt-3H₂O /10 mL distilled water)
- V Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
- VI MgCl₂ solution ; 1 M (20.33 g MgCl₂·6H₂O/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 (NH₄)₂SO₄ solution (10 mg/mL) approx. 200 U/mg at 25 °C
- IX Lactate dehydrogenase (LDH) ; (from hog muscle, Roche Diagnostics K.K., No. 127 221) 50 % glycerol solution (10 mg/mL) approx. 550 U/mg 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

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

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

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 ($\text{cm}^2/\mu\text{mol}$)
*Protein concentration ; determined by Bradford's method

REFERENCE

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

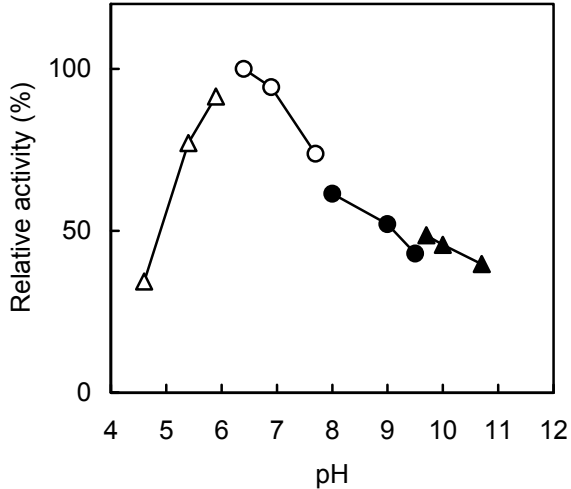


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle carbonate)

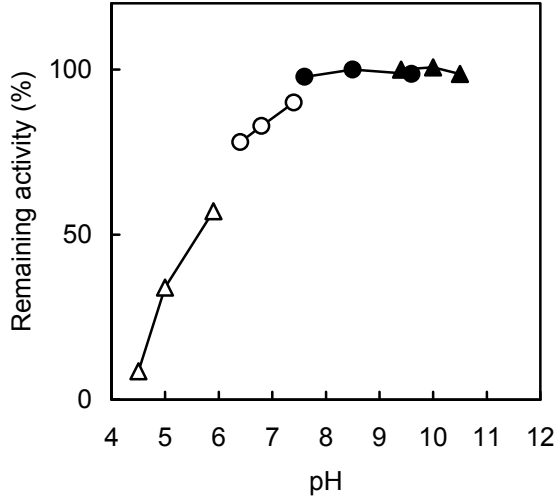


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
 Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle carbonate)

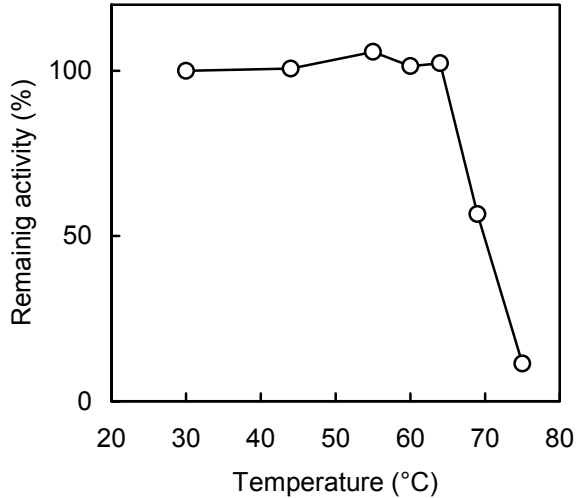


Fig. 3 Thermal stability

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

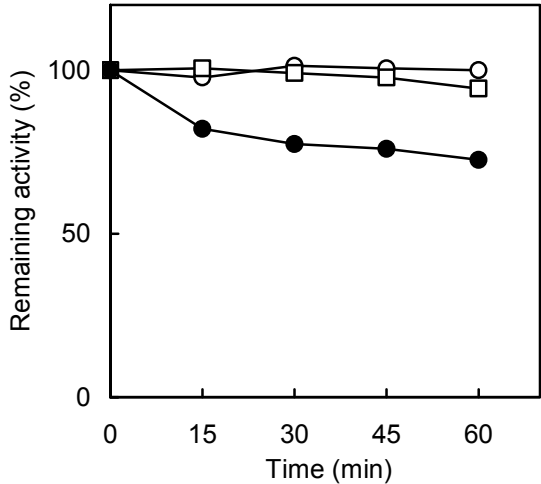


Fig. 4 Thermal stability

(treated in 0.1M Tris-HCl buffer, pH 9.0
 \circ 60 °C, \square 65 °C, \bullet 70 °C)

ALANINE DEHYDROGENASE (AlaDH)

[EC 1. 4. 1. 1]

from *Bacillus stearothermophilus*



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

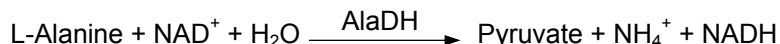
APPLICATION

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.



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 ₂ O	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 minutes (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

REFERENCE

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

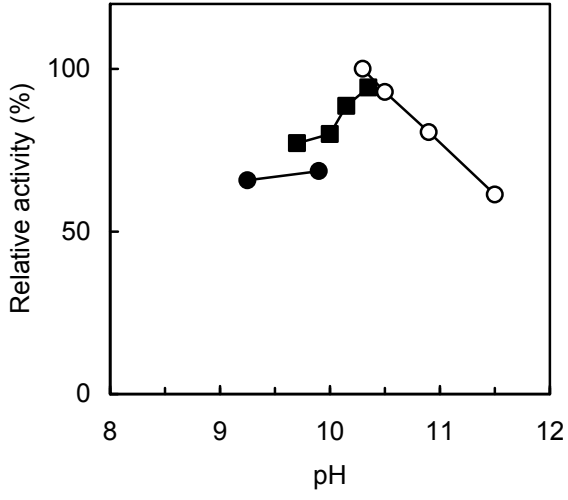


Fig. 1 pH profile

(● Tris-HCl, ■ Gly-KOH, ○ phosphate)

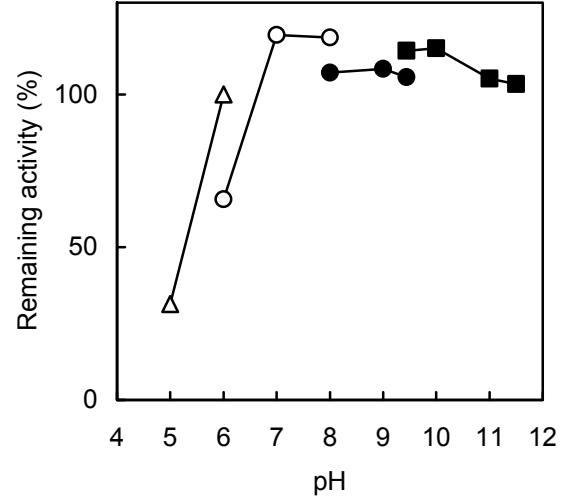


Fig. 2 pH stability

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

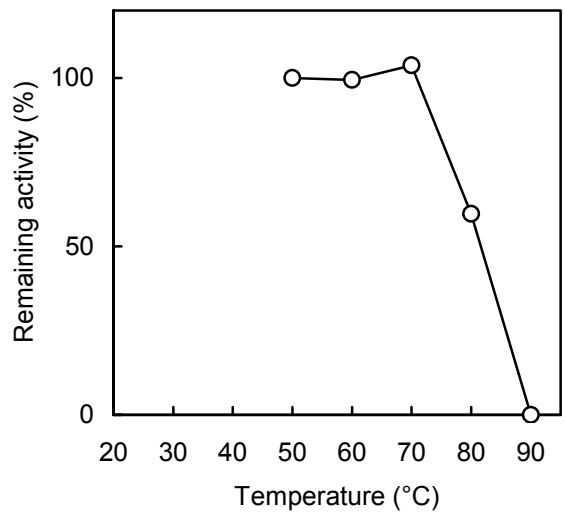


Fig. 3 Thermal stability

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

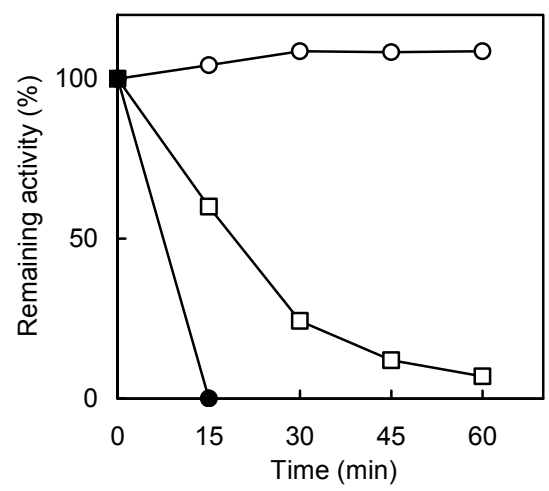


Fig. 4 Thermal stability

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

ALANINE RACEMASE (AlaR)

[EC 5. 1. 1. 1]

from *Bacillus stearothermophilus*

D-Alanine ↔ L-Alanine

SPECIFICATION

State	: Lyophilized	
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	:	

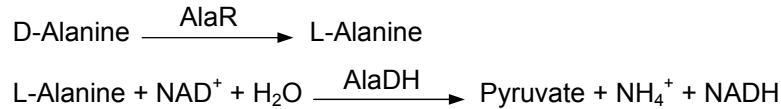
STORAGE

Stable at -20 °C at least one year

ASSAY

Principle

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



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*, Unitika Ltd., 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 ₂ O	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 minutes (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*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)

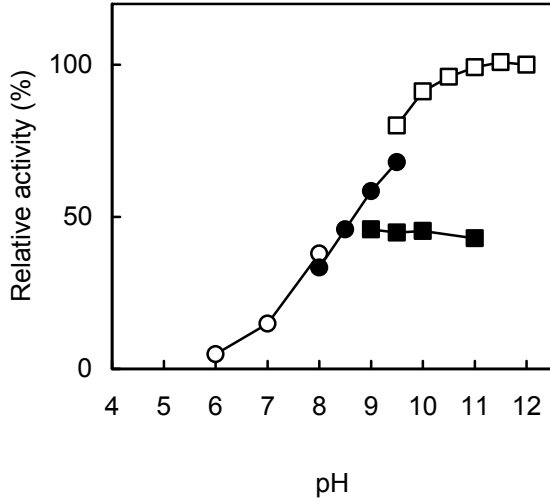


Fig. 1 pH profile

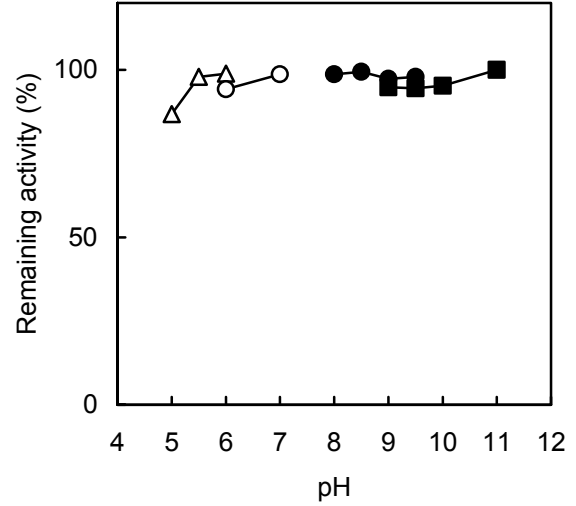


Fig. 2 pH stability

(○ phosphate, ● Tris-HCl, ■ Gly-KOH, □ NaHCO₃-NaOH)

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

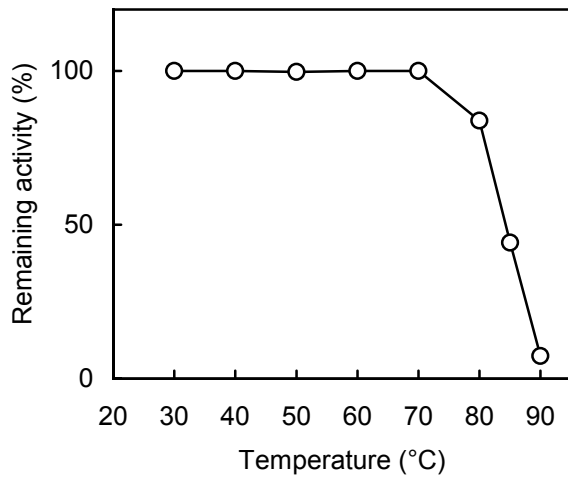


Fig. 3 Thermal stability

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

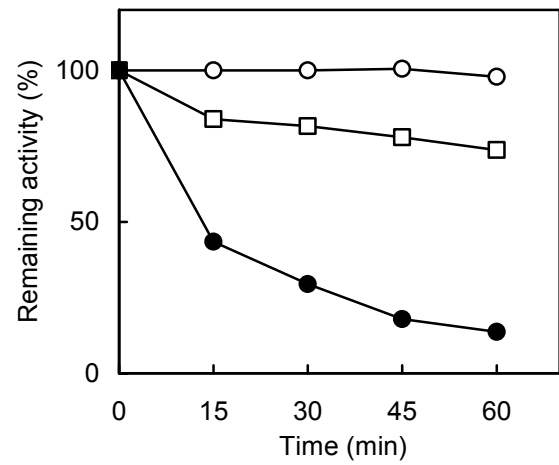


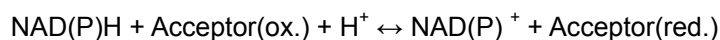
Fig. 4 Thermal stability

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

DIAPHORASE I (Di-1)

[EC 1. 6. 99. -]

from *Bacillus stearothermophilus*



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

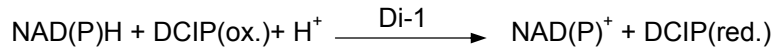
APPLICATION

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.



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·3H₂O/10 mL distilled water)
- III 2,6-Dichlorophenolindophenol (DCIP) solution ; 1.2 mM (2.0 mg DCIP sodium salt·2H₂O/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 ₂ O	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 ($\Delta\text{Abs}(\text{test})$) in linear portion of curve. Repeat the Procedure 3 using distilled water in place of enzyme solution, and $\Delta\text{Abs}(\text{blank})$ is obtained.

Calculation

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

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

d.f. ; dilution factor

19 ; millimolar extinction coefficient of DCIP ($\text{cm}^2/\mu\text{mol}$)

*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)

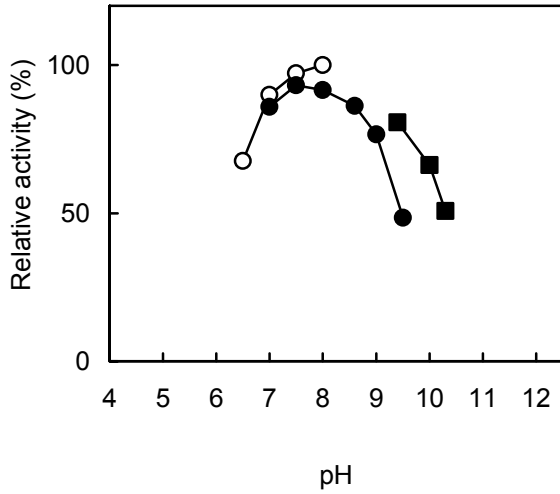


Fig. 1 pH profile

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

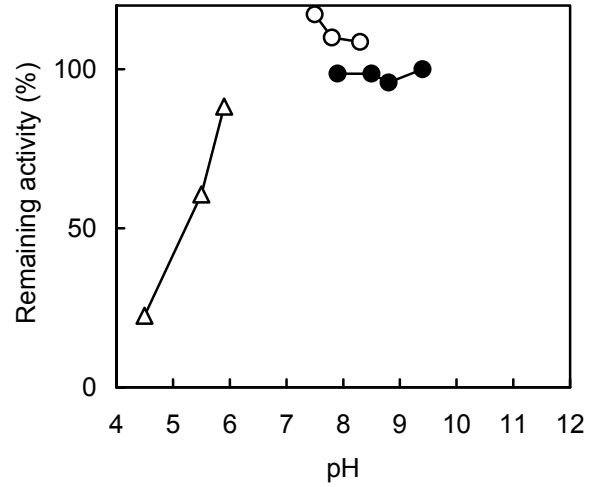


Fig. 2 pH stability

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

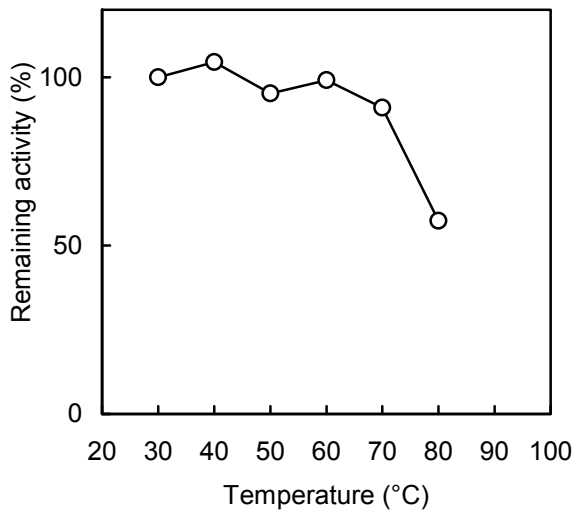


Fig. 3 Thermal stability

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

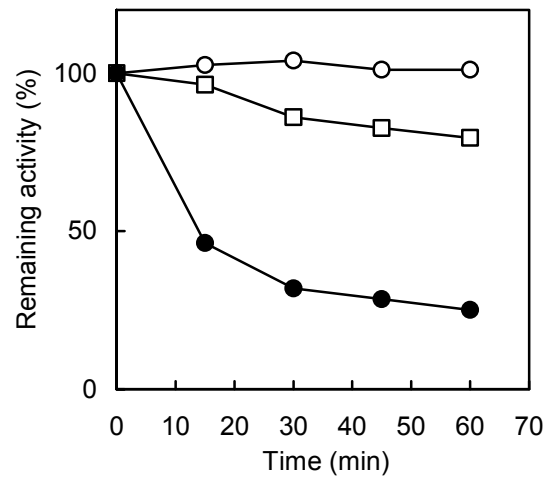


Fig. 4 Thermal stability

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

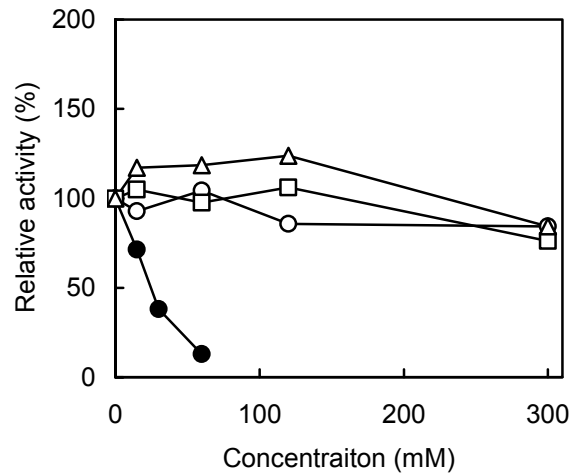


Fig. 5 Effect of various cations on the activity of DIAHORASE [EC 1.6.99.-] in the following Assay Method

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, □ MgCl₂, ● CaCl₂

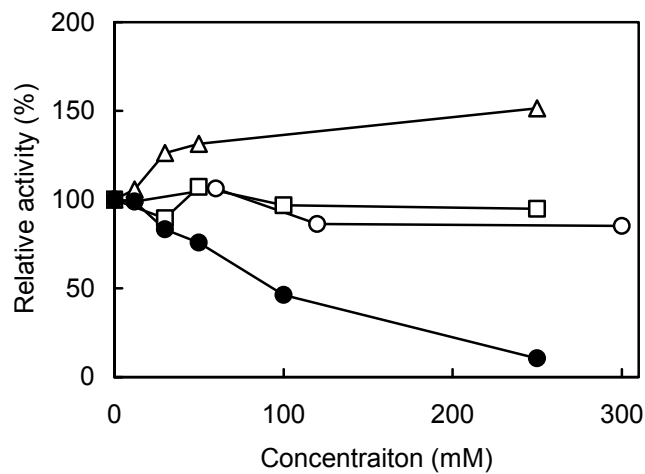


Fig. 6 Effect of various anions on the activity of DIAHORASE [EC 1.6.99.-] in the following Assay Method

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, △CH₃COONa, □ Na₂SO₄, ●NaHCO₃

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 (2 %-DMSO) 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

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)

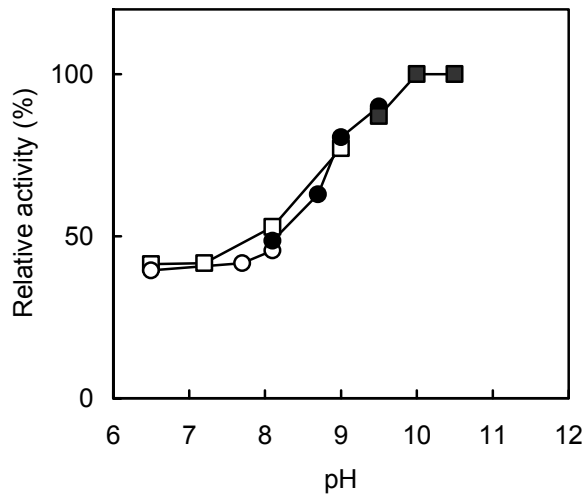


Fig. 7 pH profile

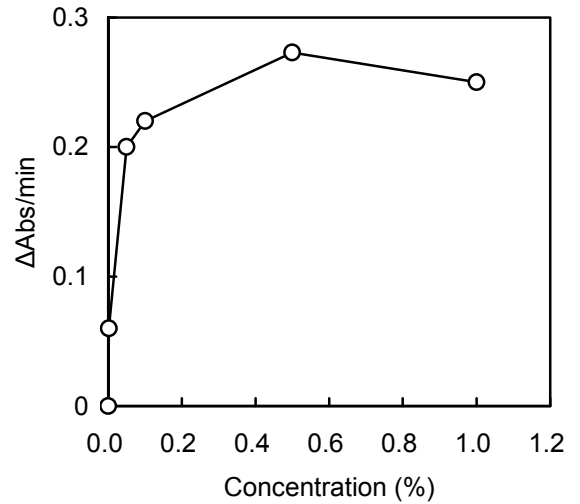
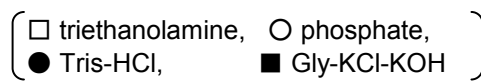


Fig. 8 Effect of Trion- X-100 on the activity of DIAPHORASE

INT (*p*-Iodonitrotetrazolium violet)

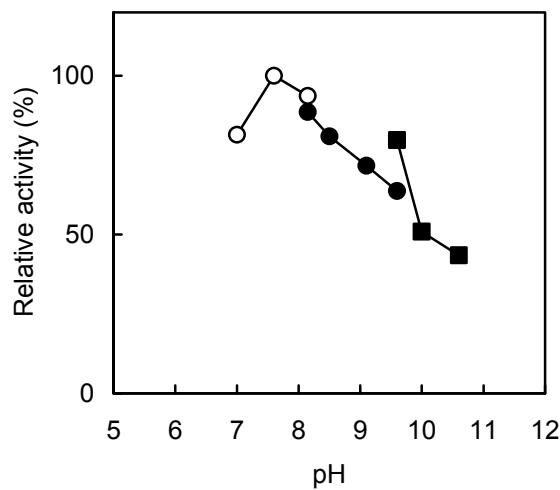


Fig. 9 pH profile

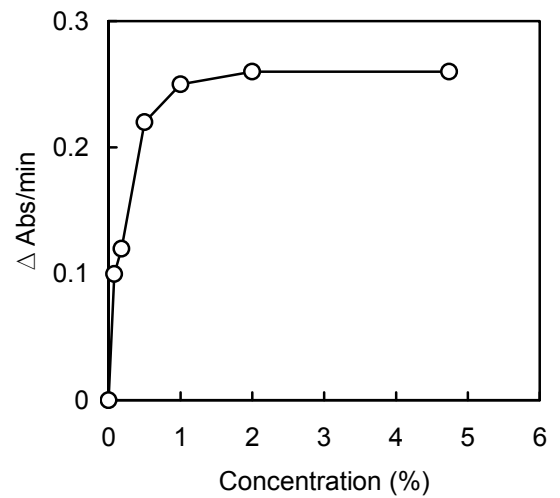
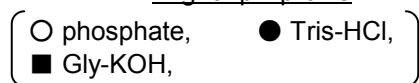
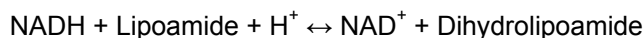


Fig. 10 Effect of BSA on the activity of DIAPHORASE

DIAPHORASE II (Di-2)

[EC 1. 8. 1. 4]

from *Bacillus stearothermophilus*



SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 150 U/mg protein	
Contaminants	: (as Diaphorase activity = 100 %)	
	Adenylate kinase	< 0.01 %
	NADH oxidase	< 0.10 %

PROPERTIES

Molecular weight	: ca. 110,000	
Subunit molecular weight	: ca. 50,000	
Optimum pH	: 6.5	(Fig. 1)
pH stability	: 6.5 - 8.0	(Fig. 2)
Isoelectric point	: 4.8	
Thermal stability	: No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	: (70 mM Potassium phosphate buffer, pH 6.5, at 30 °C)	
	Lipoate	2.0 mM
	NADH	0.01 mM
Substrate specificity	: Lipoate	100 %
	Lipoamide	43 %
	2,6-Dichlorophenolindophenol (DCIP)	20 %
	Nitro blue tetrazolium (NBT)	110 %
	NADH	100 %
	NADPH	1 %
Effectors	: cations and anions	(Fig. 5, 6)

STORAGE

Stable at -20 °C for at least one year

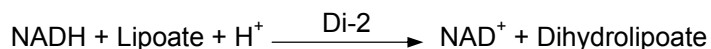
APPLICATION

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

ASSAY

Principle

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



Unit Definition

One unit of activity is defined as the amount of Di-2 that forms 1 μmol of NAD^+ per minute at 30 °C.

Solutions

- I Buffer solution ; 100 mM Potassium phosphate, pH 6.5
- II EDTA solution ; 27 mM (0.100 g EDTA disodium salt·2H₂O/10 mL distilled water)
- III Lipoate solution ; 60 mM (0.124 g DL- α -lipoate/10 mL 0.1 M K₂HPO₄ solution)
- IV BSA solution ; (100 mg BSA/10 mL distilled water)
- V NADH solution ; 13.1 mM (0.100 g NADH disodium salt·3H₂O/10 mL distilled water)
- VI NAD⁺ solution ; 30 mM (0.215 g NAD⁺·3H₂O/10 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 3.00 mL of reaction mixture into a cuvette.

Solution I	21.15mL	SolutionIV	2.10mL
Solution II	0.90mL	Solution V	0.45mL
SolutionIII	5.10mL	SolutionVI	0.30mL
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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

REFERENCE

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

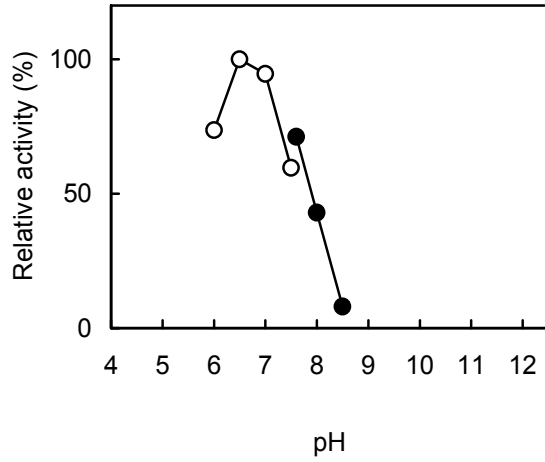


Fig. 1 pH profile

(○ phosphate, ● Tris-HCl)

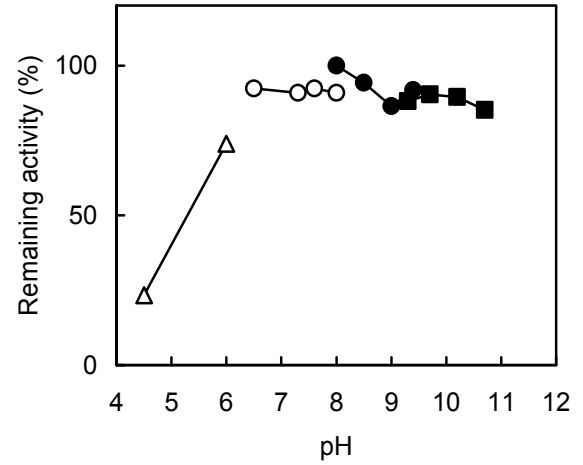


Fig. 2 pH stability

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

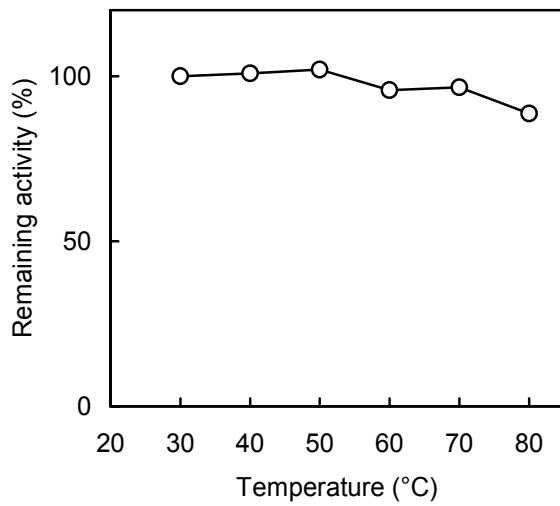


Fig. 3 Thermal stability

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

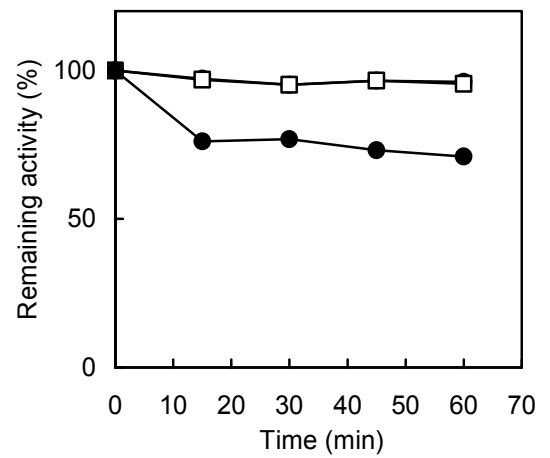


Fig. 4 Thermal stability

(treated in 0.1 M potassium phosphate buffer, pH 7.5
○ 60 °C, □ 70 °C, ● 80 °C)

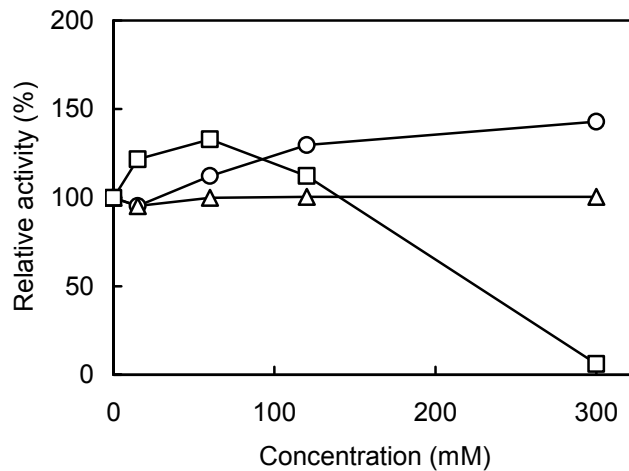


Fig. 5 Effect of various cations on the activity of DIAHORASE [EC 1.8.1.4] in the following Assay Method

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, 0.01 mL of enzyme solution was added to the reaction mixture and the activity of DIAPHORASE was measured.

○ NaCl, △ KCl, □ MgCl₂,

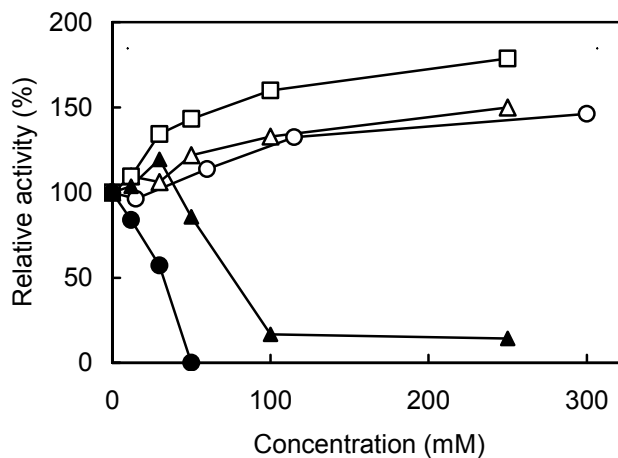


Fig. 6 Effect of various anions on the activity of DIAHORASE [EC 1.8.1.4] in the following Assay Method

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, △ CH₃COONa, □ Na₂SO₄,
● NaHCO₃, ▲ NaH₂PO₄

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-HCl buffer, pH 8.5, at 30 °C)	
	Glucose	0.1 mM
	ATP	0.05 mM
Substrate specificity	: D-Glucose	100 %
	D-Mannose	0 %
	D-Fructose	0 %

STORAGE

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

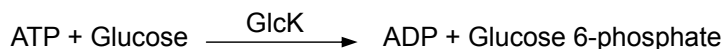
APPLICATION

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.



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·3H₂O)/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
- III MgCl₂ solution ; 1 M (20.33 g MgCl₂·6H₂O/100 mL distilled water)
- IV NADP⁺ solution ; 22.5 mM (0.188 g NADP⁺ sodium salt·4H₂O)/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 (NH₄)₂SO₄ 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
Solution III	0.60 mL	Solution VI	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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADPH (cm²/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)

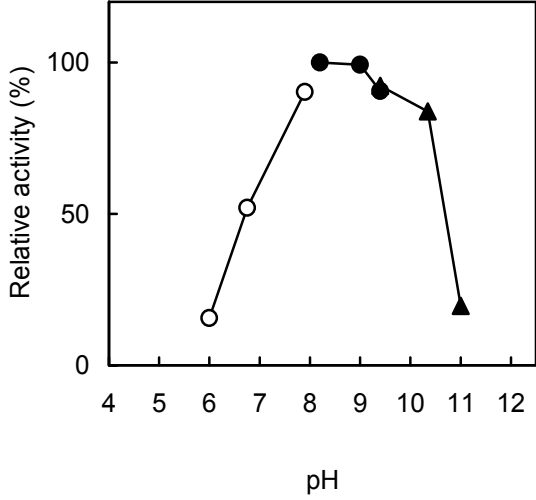


Fig. 1 pH profile

(○ phosphate, ● Tris-HCl, ▲ carbonate)

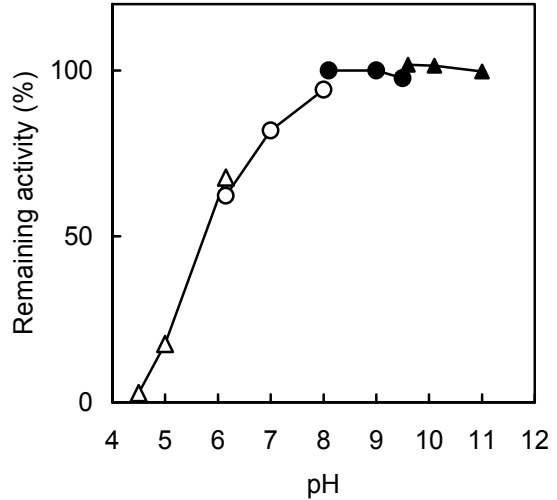


Fig. 2 pH stability

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

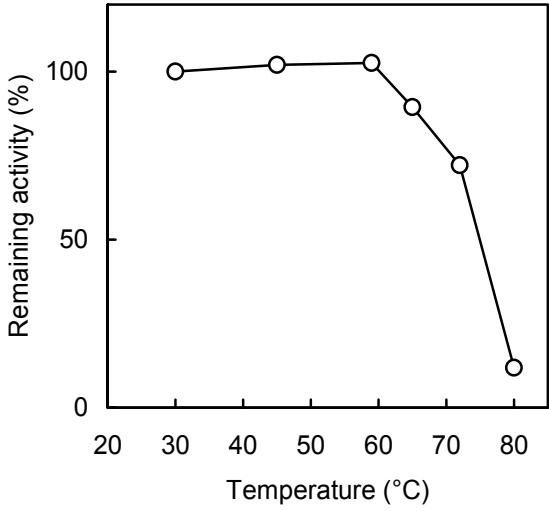


Fig. 3 Thermal stability

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

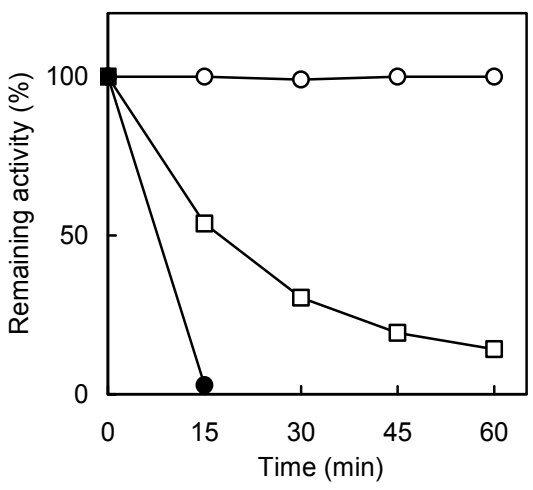


Fig. 4 Thermal stability

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

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH)

[EC 1.1.1.49]

from *Bacillus stearothermophilus*



SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 150 U/mg protein	
Contaminants	: (as 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. 195,000	
Subunit molecular weight	: ca. 53,000	
Optimum pH	: 8.7	(Fig. 1)
pH stability	: 7.5 - 11.0	(Fig. 2)
Isoelectric point	: 6.5 - 6.8	
Optimum temperature	: 70 °C	
Thermal stability	: No detectable decrease in activity up to 60 °C.	(Fig. 3, 4)
Michaelis constants	: (84 mM Tris-HCl buffer, pH 9.0, at 30 °C)	
	Glucose 6-phosphate	0.16 mM
	NADP ⁺	0.016 mM
	NAD ⁺	1.64 mM
Substrate specificity	: Glucose 6-phosphate	100 %
	2-Deoxyglucose 6-phosphate	0 %
	Mannose 6-phosphate	0 %
	Fructose 6-phosphate	0 %
	Glucose 1-phosphate	0 %
	Glucosamine 6-phosphate	0 %
	6-Phosphogluconate	0 %
	: NADP ⁺	100 %
	NAD ⁺	115 %
Effecters	: cations and anions	(Fig. 5, 6)

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.



Unit Definition

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

Solutions

- I Buffer solution ; 100 mM Tris-HCl, pH 9.0
- II MgCl_2 solution ; 1 M (20.33 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ /100 mL distilled water)
- III NADP^+ solution ; 22.5 mM (0.188 g NADP^+ sodium salt- $4\text{H}_2\text{O}$ /10 mL distilled water)
- IV Glucose 6-phosphate (G6P) solution ; 33 mM (0.112 g G6P disodium salt- $2\text{H}_2\text{O}$ /10 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 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	25.20mL	Solution III	1.20mL
Solution II	1.20mL	Solution IV	2.40mL
2. Incubate at 30 °C for about 3minutes.
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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADPH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

REFERENCE

1. Okuno. H., Nagata, K., and Nakajima, H.; *J. Appl Biochem.*, 7.192 (1985)

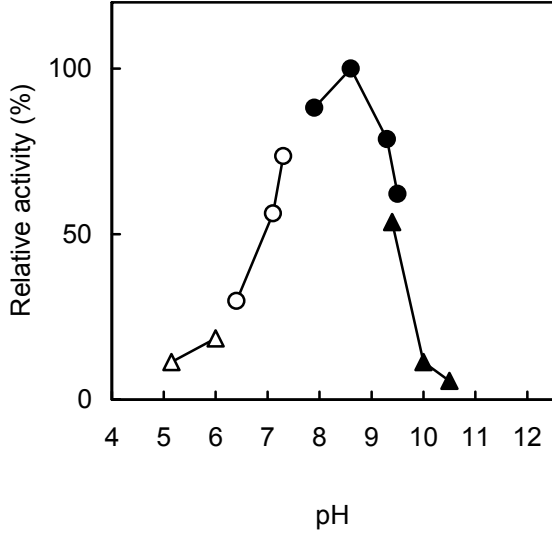


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle carbonate)

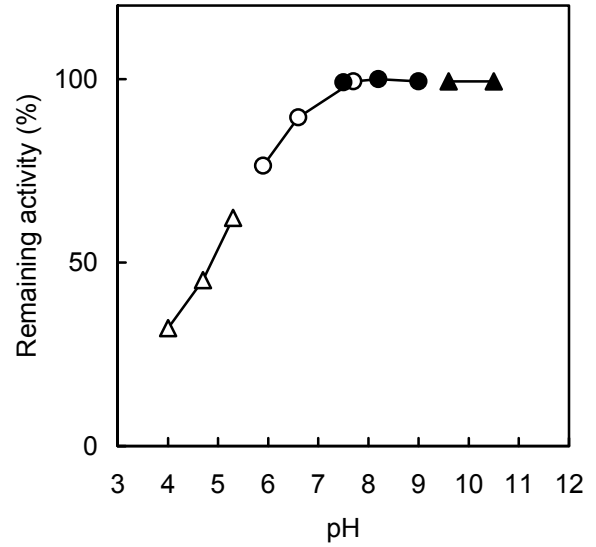


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
 Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle carbonate)

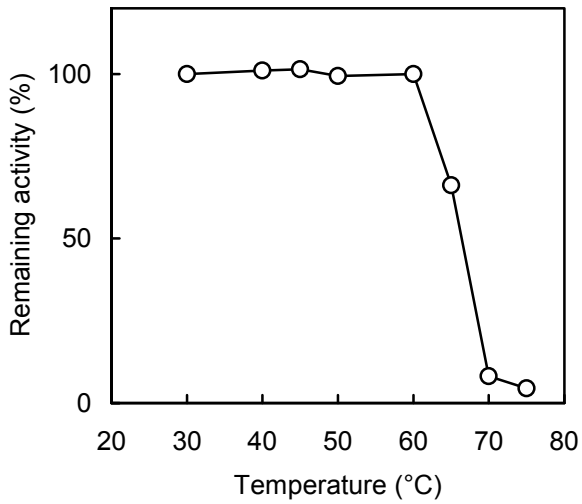


Fig. 3 Thermal stability

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

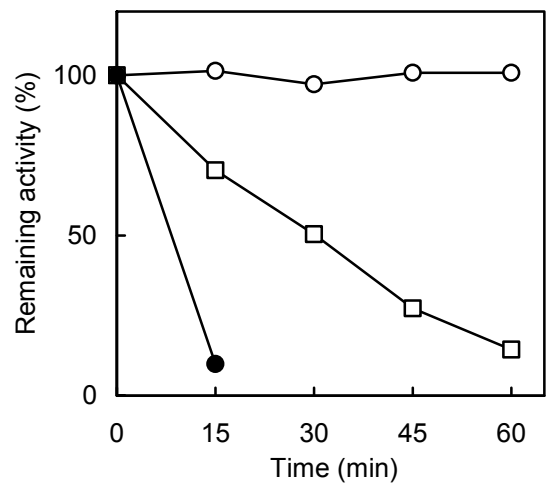


Fig. 4 Thermal stability

(treated in 0.1 M Tris-HCl buffer, pH 8.9
 \circ 60 °C, \square 65 °C, \bullet 70 °C)

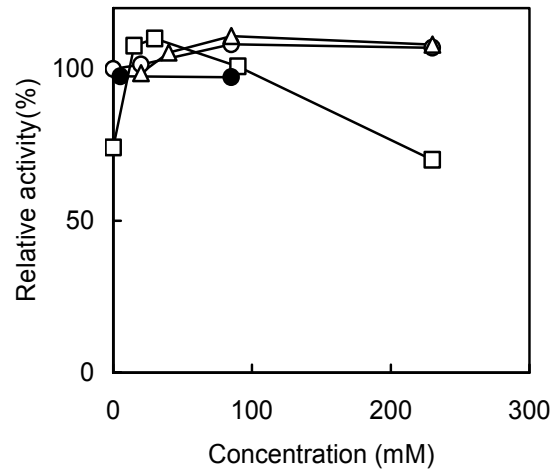


Fig. 5 Effect of various cations on the activity of Glucose-6-phosphate dehydrogenase in the following *Assay Method*

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, 0.01 mL of enzyme solution was added to the reaction mixture and the activity of Glucose-6-phosphate dehydrogenase was measured.

○ NaCl, △ KCl, □ MgCl₂, ● CaCl₂

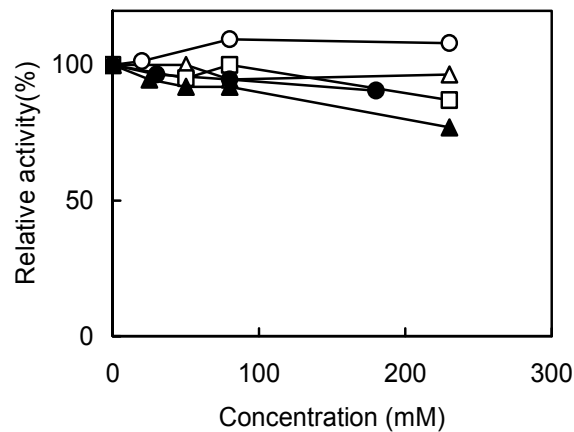


Fig. 6 Effect of various anions on the activity of Glucose-6-phosphate dehydrogenase in the following *Assay Method*

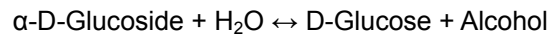
Measurement : 0.30 mL of each anion solution and 3.00 mL of assay mixture were mixed, and incubated at 30 °C for about 3 minutes. After incubation, 0.01 mL of enzyme solution was added to the reaction mixture and the activity of Glucose-6-phosphate dehydrogenase was measured.

○ NaCl, △ CH₃COONa, □ Na₂SO₄,
● NaHCO₃, ▲ NaH₂PO₄

α -GLUCOSIDASE (α -Glu)

[EC 3.2.1.20]

from *Bacillus stearothermophilus*



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 %
	Maltase	177 %
	Phenyl- α -glucopyranoside	59 %

STORAGE

Stable at -20 °C for at least one year

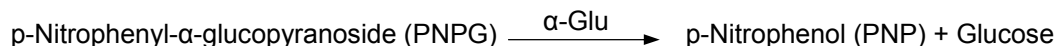
APPLICATION

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.



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

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

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

d.f. ; dilution factor

18.1 ; millimolar extinction coefficient of PNP ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

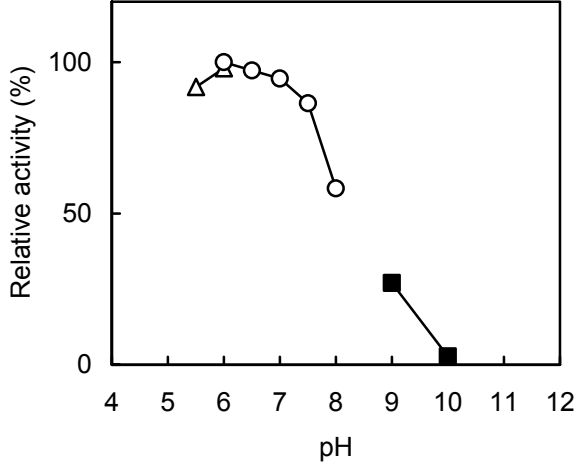


Fig. 1 pH profile

(Δ acetate, \circ phosphate, \blacksquare Gly-NaOH)

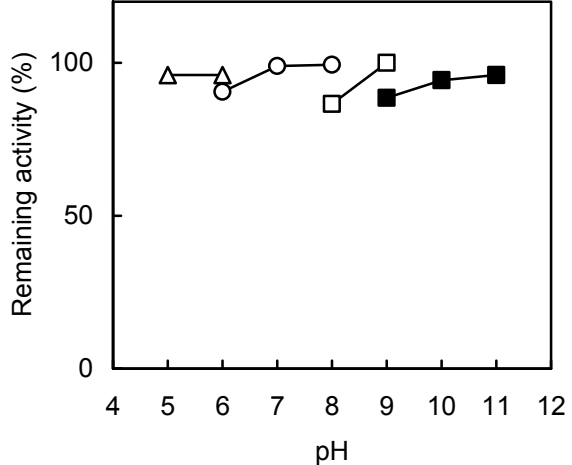


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
 Δ acetate, \circ phosphate, \square TEA-NaOH, \blacksquare Gly-NaOH)

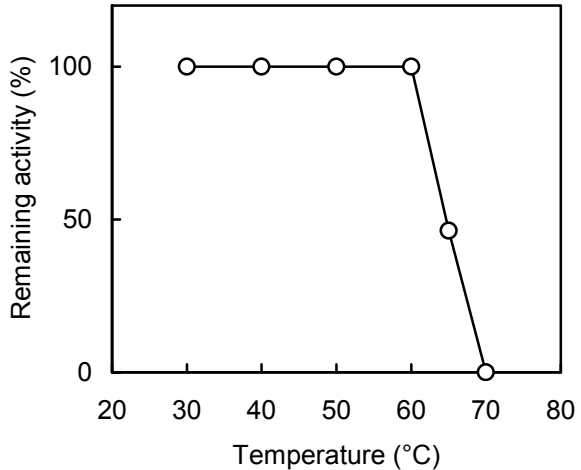


Fig. 3 Thermal stability

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

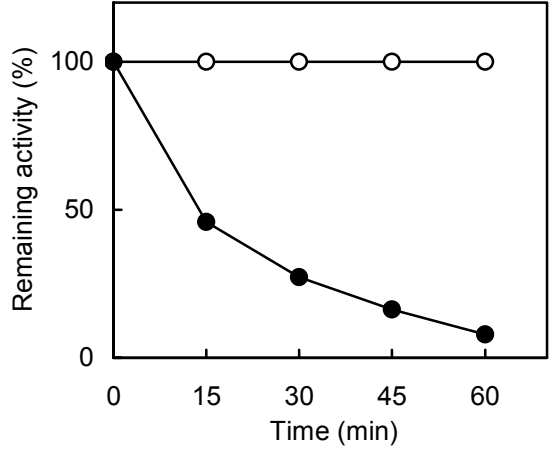


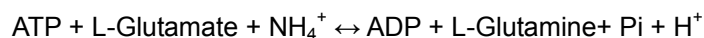
Fig. 4 Thermal stability

(treated for in 0.1M potassium phosphate buffer, pH 8.0
 \circ 60 °C, \blacksquare 65 °C)

GLUTAMINE SYNTHETASE (GS)

[EC 6. 3. 1. 2]

from *Bacillus stearothermophilus*



SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 10 U/mg protein	
Contaminants	: (as GS activity = 100 %)	
	ATPase	< 0.01 %
	Glutamate dehydrogenase	< 0.01 %

PROPERTIES

Molecular weight	: ca. 510,000	
Subunit molecular weight	: ca. 43,000	
Optimum pH	: 7.0	(Fig. 1)
pH stability	: 6.5 - 8.0	(Fig. 2)
Thermal stability	: No detectable decrease in activity up to 55 °C.	(Fig. 3, 4)
Michaelis constants	: (70 mM Imidazole-HCl buffer, 50 mM MgCl ₂ , pH 7.2, at 30 °C)	
	L-Glutamate	12 mM
	ATP	1.3 mM
	NH ₄ ⁺	0.08 mM
Substrate specificity	: L-Glutamate	100 %
	D-Glutamate	3 %
	α-Methylglutamate	4 %
	L-Glutarate	0 %
	L-Aspartate	0 %
Effectors	: cations (Mg ²⁺ , Mn ²⁺ etc.)	

STORAGE

Stable at -20 °C for at least one year

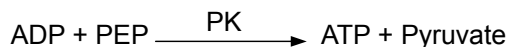
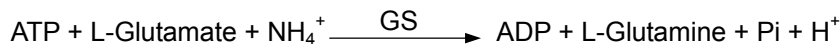
APPLICATION

The enzyme is useful for determination of L-glutamate or ammonia.

ASSAY

Principle

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



Unit Definition

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

Solutions

- I Buffer solution ; 100 mM Imidazole-HCl, pH 7.2
- II L- Glutamate solution ; 250 mM (4.678 g L-glutamate sodium salt/100 mL distilled water)
- III ATP solution ; 100 mM (0.605 g ATP disodium salt-3H₂O/(8.2 mL distilled water + 1.8 mL 1 N -NaOH))
- IV NH₄Cl solution ; 1 M (5.349 g ammonium chloride/100 mL distilled water)
- V Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
- VI NADH solution ; 13.1 mM (0.100 g NADH disodium salt-3H₂O/10 mL distilled water)
- VII MgCl₂ solution ; 1M (20.33 g MgCl₂·6H₂O/100 mL distilled water)
- VIII KCl solution ; 2.5 M (18.64 g KCl/100 mL distilled water)
- IX Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M (NH₄)₂SO₄ solution (10 mg/mL) approx. 200 U/mg at 25 °C
- X Lactate dehydrogenase (LDH) ; (from hog muscle, Roche Diagnostics K.K., No. 127 221) 50 % glycerol solution (25 mg/2.5 mL) approx. 550 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 imidazole-HCl buffer, pH 7.2.

Procedure

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

Solution I	21.01mL	SolutionVI	0.60mL
Solution II	3.60mL	SolutionVII	1.50mL
SolutionIII	1.20mL	SolutionVIII	1.20mL
SolutionIV	0.15mL	SolutionIX	0.12mL
Solution V	0.56mL	SolutionX	0.06mL
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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)
*Protein concentration ; determined by Bradford's method

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1. Wedler, F.C., and Hoffmann, F.C.; *Biochemistry*, **13**, 3207 (1974)
2. Hachimori, A., Matsunaga, A., Shimizu, M., Samejima, T., and Nosoh, Y.; *Biochim. Biophys. Acta*, **350**, 461 (1974)

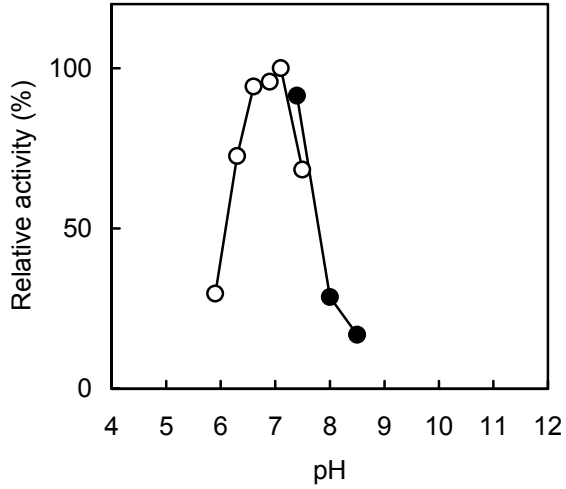


Fig. 1 pH profile

(○ imidazole-HCl, ● Tris-HCl)

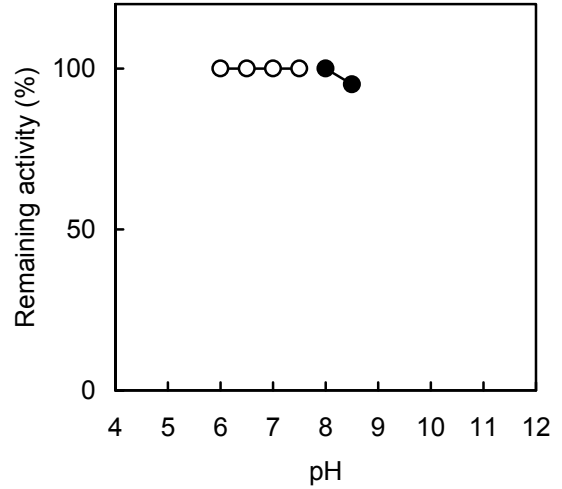


Fig. 2 pH stability

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

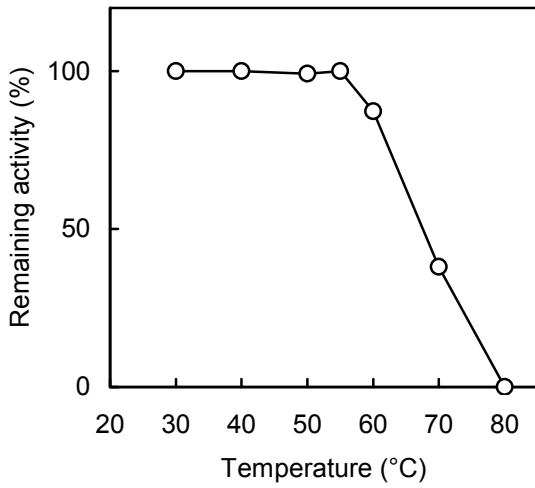


Fig. 3 Thermal stability

(treated for 15 min in 0.1 M imidazole-HCl buffer, pH 7.2)

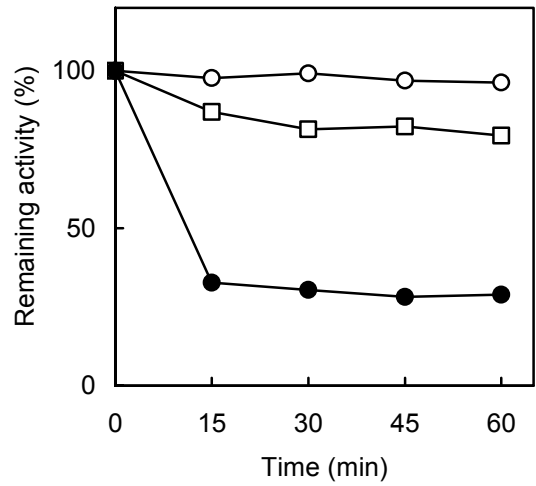


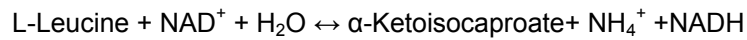
Fig. 4 Thermal stability

(treated in 0.1 M imidazole-HCl buffer, pH 7.2
○ 55 °C, □ 60 °C, ● 70 °C)

LEUCINE DEHYDROGENASE (LeuDH)

[EC 1. 4. 1. 9]

from *Bacillus stearothermophilus*



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

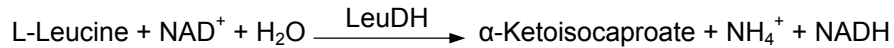
APPLICATION

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.



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 ₂ O	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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

REFERENCE

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

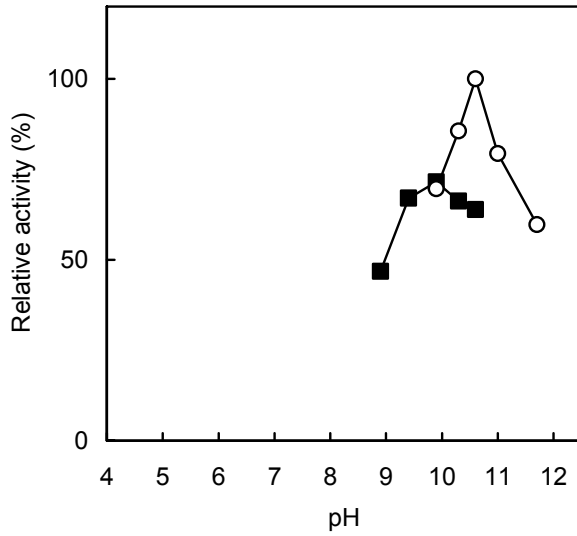


Fig. 1 pH profile

[■ Gly-KOH, ○ phosphate]

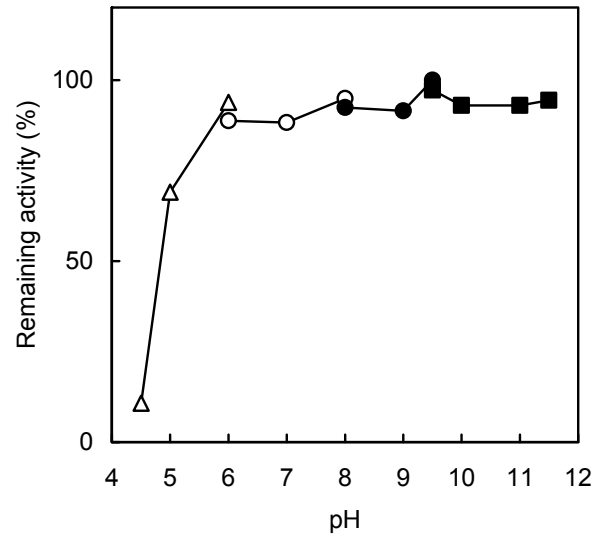


Fig. 2 pH stability

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

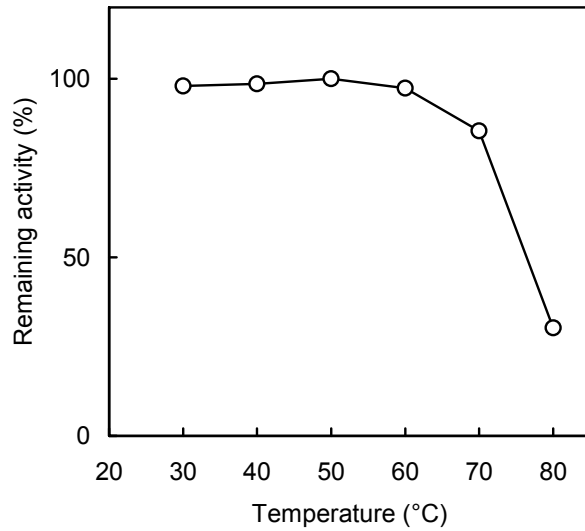


Fig. 3 Thermal stability

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

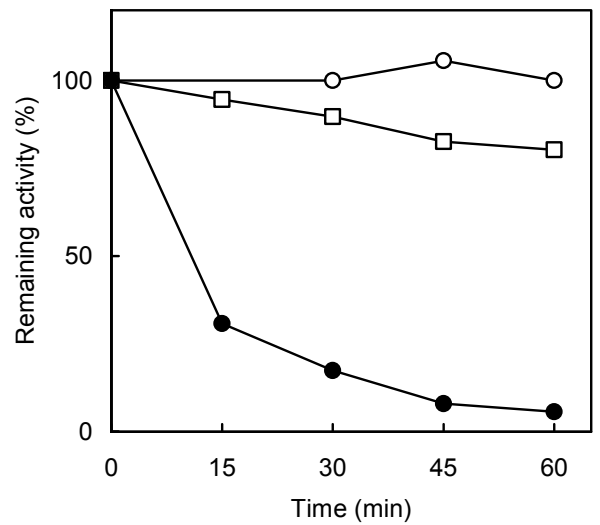


Fig. 4 Thermal stability

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

PHOSPHOFRUCTOKINASE (PFK)

[EC 2. 7. 1. 11]

from *Bacillus stearothermophilus*



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) ₂ SO ₄	
Inhibitors	: PEP, Citrate	

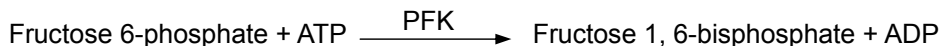
STORAGE

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.



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-HCl, pH 9.0
- II ATP solution ; 100 mM (0.605 g ATP disodium salt·3H₂O/(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·3H₂O/10mL distilled water)
- V Fructose 6-phosphate (F6P) solution ; 500 mM (1.55 g F6P disodium salt/10 mL distilled water)
- VI KCl solution ; 2.5 M (16.64g KCl/100 mL distilled water)
- VII MgSO₄ solution ; 100 mM (2.47 g MgSO₄·7H₂O/100 mL distilled water)
- VIII Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M (NH₄)₂SO₄ solution (10 mg/mL) approx. 200 U/mg at 25 °C
- IX Lactate dehydrogenase (LDH) ; (from hog muscle, Roche Diagnostics K.K., No. 127 221) 50 % glycerol solution (2.5 mg/2.5 mL) approx. 550 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 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
Solution V	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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)
*Protein concentration ; determined by Bradford's method

REFERENCE

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

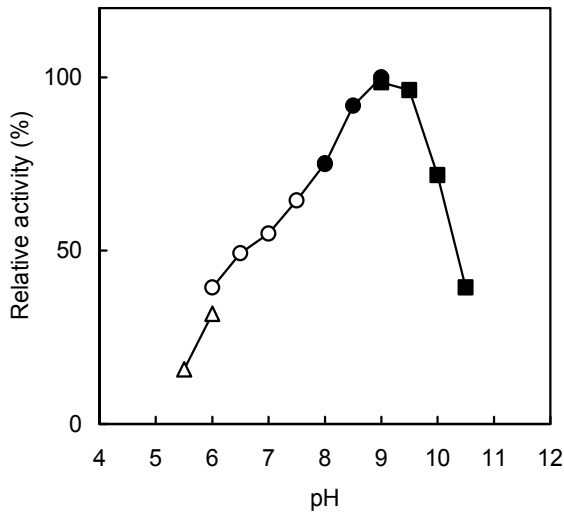


Fig. 1 pH profile

(
 Δ acetate, ○ phosphate,
 ● Tris-HCl, ■ Gly-KOH
)

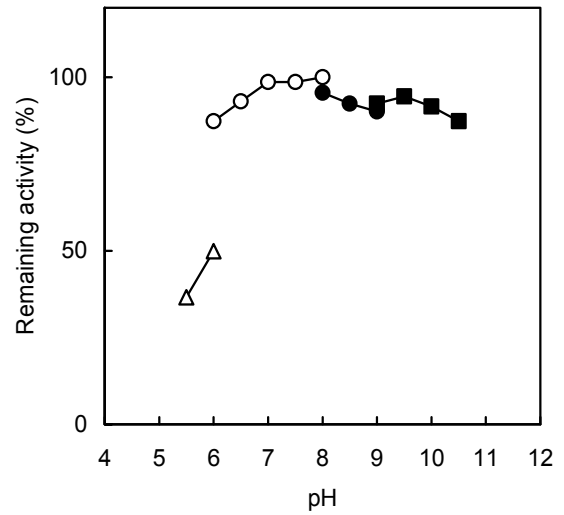


Fig. 2 pH stability

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

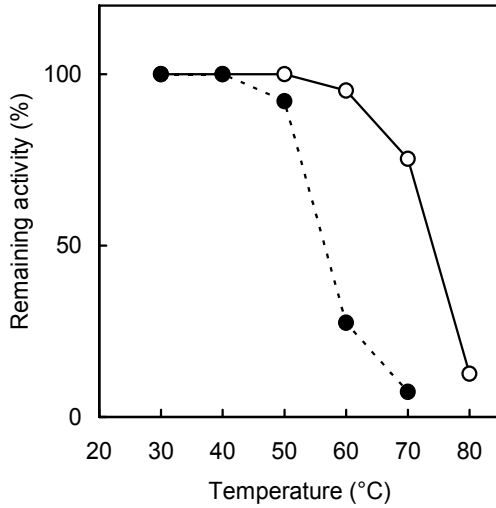


Fig. 3 Thermal stability

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

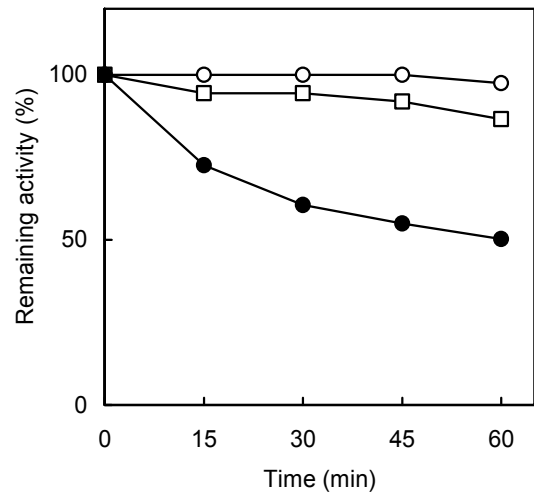


Fig. 4 Thermal stability

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

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-HCl buffer, pH 9.0, at 30 °C)	
	Fructose 6-phosphate	0.27 mM

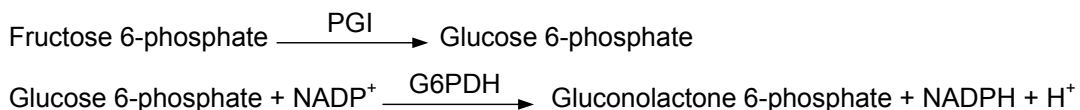
STORAGE

Stable at -20 °C for at least one year

ASSAY

Principle

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



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·4H₂O/10 mL distilled water)
- IV Glucose-6-phosphate dehydrogenase (G6PDH) ; (from yeast, Roche Diagnostics K.K., No. 127 671) suspension in 3.2 M (NH₄)₂SO₄ 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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADPH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

REFERENCE

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

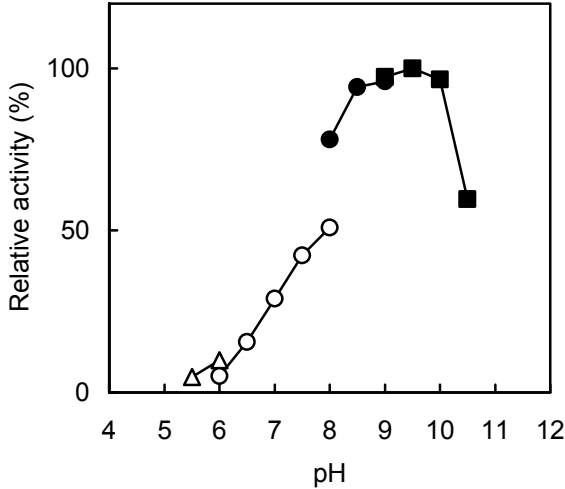


Fig. 1 pH profile

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

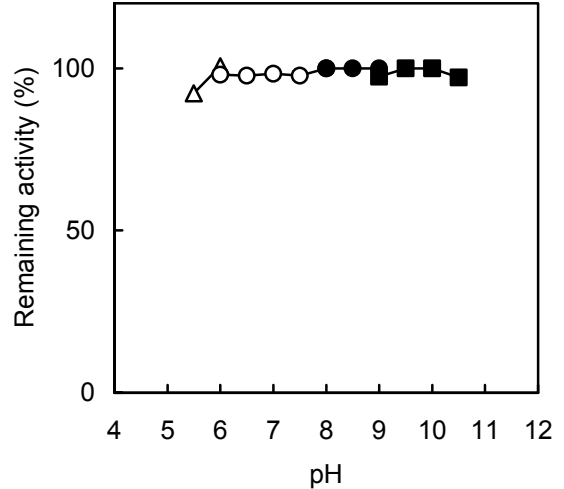


Fig. 2 pH stability

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

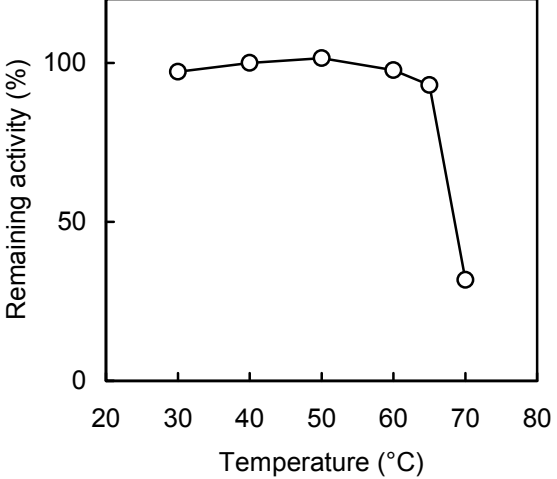


Fig. 3 Thermal stability

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

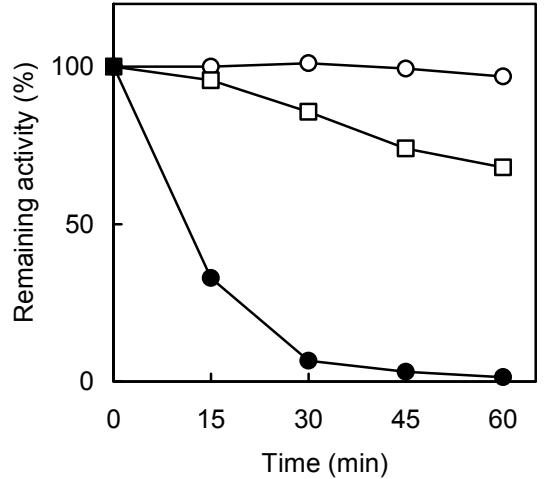


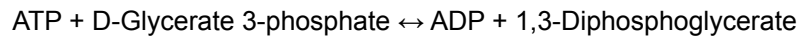
Fig. 4 Thermal stability

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

PHOSPHOGLYCERATE KINASE (PGK)

[EC 2. 7. 2. 3]

from *Bacillus stearothermophilus*



SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 400 U/mg protein	
Contaminants	: (as PGK activity = 100 %)	
	Glyceraldehyde-3-phosphate dehydrogenase	< 0.01 %
	Glycerate-3-phosphate dehydrogenase	< 0.01 %
	Adenylate kinase	< 0.01 %
	Triosephosphate isomerase	< 0.01 %
	NADH oxidase	< 0.01 %

PROPERTIES

Molecular weight	: ca. 40,000	
Optimum pH	: 7	(Fig. 1)
pH stability	: 7.5 - 10.5	(Fig. 2)
Isoelectric point	: 4.4	
Thermal stability	: No detectable decrease in activity up to 50 °C.	(Fig. 3, 4)
Michaelis constants	: (74 mM Triethanolamine-HCl buffer, pH 7.2, at 30 °C)	
	Glycerate 3-phosphate	0.7 mM
	ATP	0.7 mM

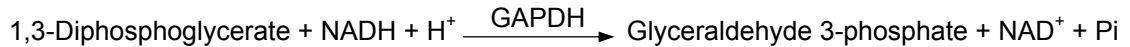
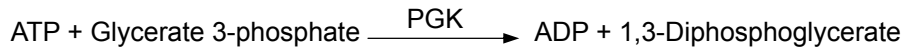
STORAGE

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.



Unit Definition

One unit of activity is defined as the amount of PGK that forms 1 μmol of 1,3-diphosphoglycerate per minute at 30 °C.

Solutions

- I Buffer solution ; 100 mM Triethanolamine-HCl, pH 7.2
- II NADH solution ; 13.1 mM (0.100 g NADH disodium salt·3H₂O/10 mL distilled water)
- III ATP solution ; 100 mM (0.605 g ATP disodium salt·3H₂O/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
- IV Glycerate 3-phosphate (G3P) solution ; 100 mM (0.126 g G3P trisodium salt/5 mL distilled water)
- V MgCl₂ solution ; 1M (20.33 g MgCl₂·6H₂O/100 mL distilled water)
- VI Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ; 1000 U/mL (from rabbit muscle, Sigma-Aldrich Co., No. G2267, 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 Tris-HCl buffer, pH 8.5.

Procedure

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

Solution I	22.20mL	SolutionIV	3.90mL
Solution II	0.45mL	Solution V	0.36mL
Solution III	3.00mL	Solution VI	0.09mL
- Incubate at 30 °C for about 3 minutes.
- Add 0.01 mL of enzyme solution into the cuvette and mix.
- Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

REFERENCE

- Suzuki, K., and Imahori, K.; *J. Biochem.*, **76**, 771 (1974)

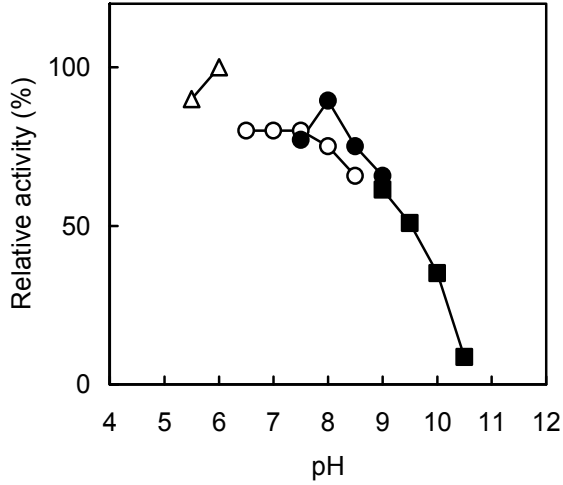


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
● Tris-HCl, ■ Gly-KOH)

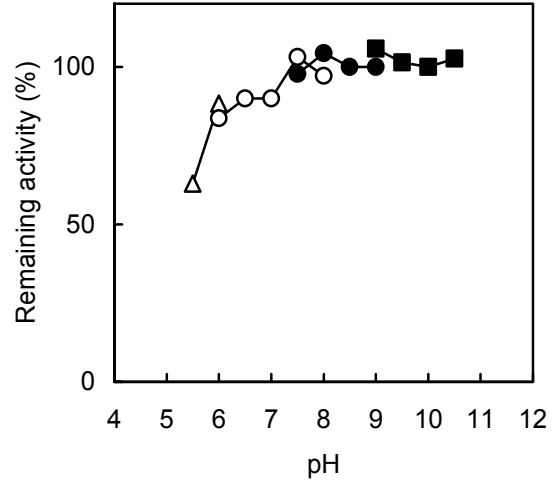


Fig. 2 pH stability

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

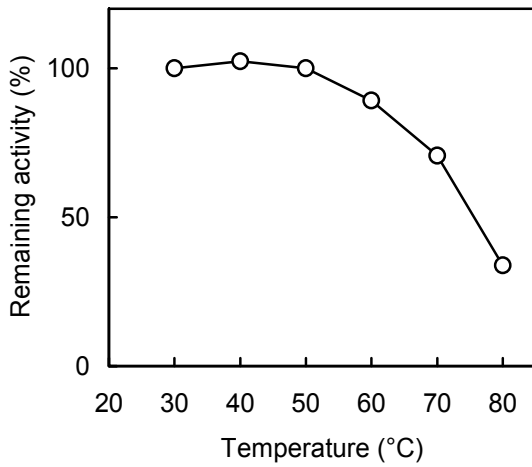


Fig. 3 Thermal stability

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

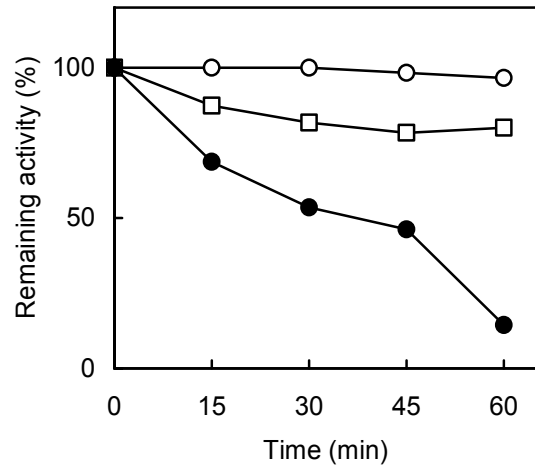


Fig. 4 Thermal stability

(treated in 50 mM Tris-HCl buffer, pH 8.0
 \circ 50 °C, \square 60 °C, \bullet 70 °C)

PHOSPHOTRANSACETYLASE (PTA)

[EC 2. 3. 1. 8]

from *Bacillus stearothermophilus*



SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of CoA or acetate.

ASSAY

Principle

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



Unit Definition

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

Solutions

- 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

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

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

d.f. ; dilution factor

4.44 ; differential millimolar extinction coefficient between acetyl-CoA and CoA ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

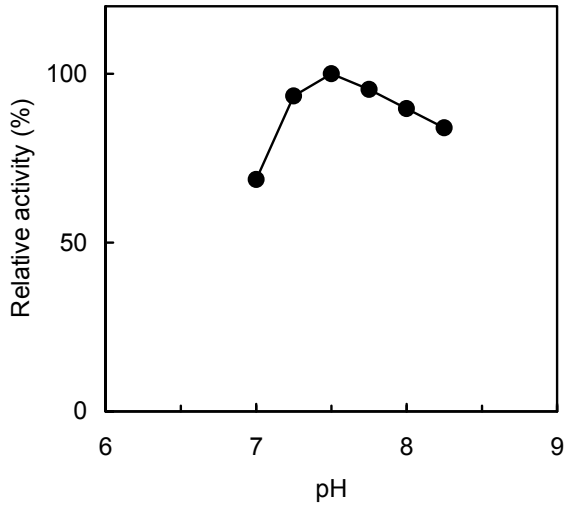


Fig. 1 pH profile
[● Tris-HCl]

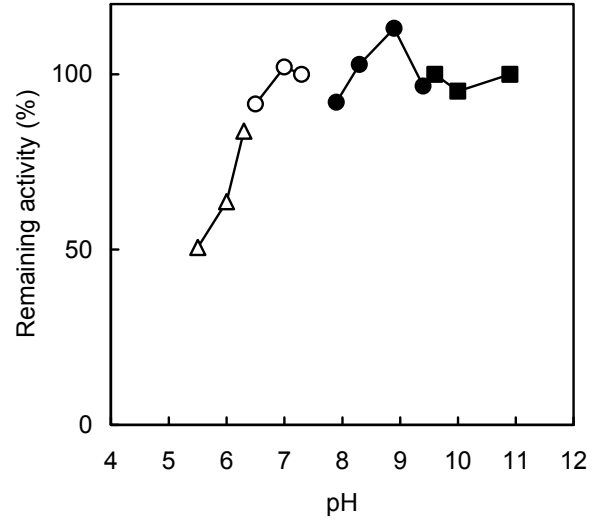


Fig. 2 pH stability
(treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH)

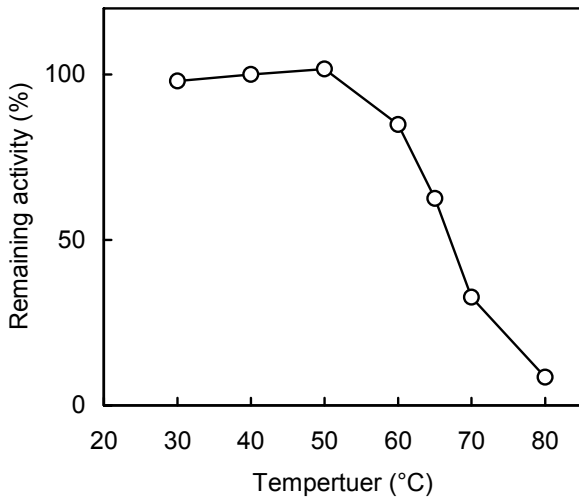


Fig. 3 Thermal stability
(treated for 15 min in 50 mM Tris-HCl buffer, pH 8.0)

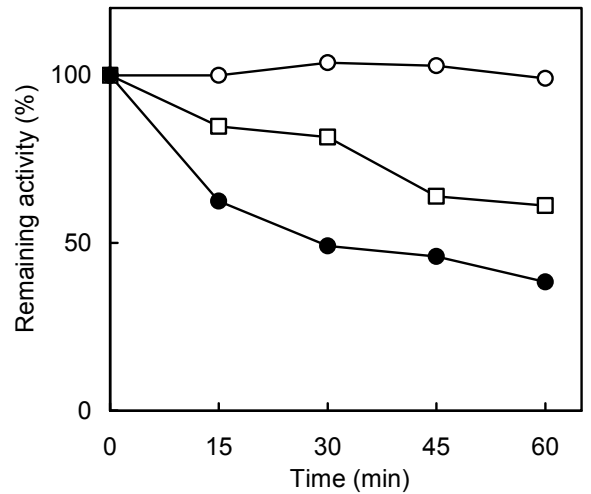
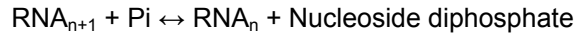


Fig. 4 Thermal stability
(treated in 50 mM Tris-HCl buffer, pH 8.0
○ 50 °C, □ 60 °C, ● 65 °C)

POLYNUCLEOTIDE PHOSPHORYLASE (PNPase)

[EC 2. 7. 7. 8]

from *Bacillus stearothermophilus*



FOR DEPOLYMERIZATION REACTION

SPECIFICATION

State	: Lyophilized	
Specific activity	: more than 2,000 U/mg protein	
Contaminants	: (as PNPase activity = 100 %)	
	Ribonuclease	< 0.0001 %

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-HCl buffer, pH 9.5, at 60 °C)	
	Poly A	0.27 mM**
	KH ₂ PO 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

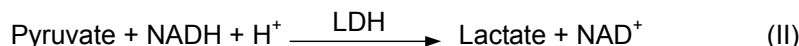
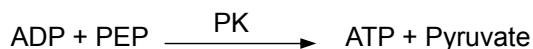
APPLICATION

The enzyme is useful for the preparation of polyribonucleotide (see p. 70).

ASSAY

Principle

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



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 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ + 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 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ + 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 $\cdot 3\text{H}_2\text{O}$ /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 $(\text{NH}_4)_2\text{SO}_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C
- VIII Lactate dehydrogenase (LDH) ; (from hog muscle, Roche Diagnostics K.K., No. 127 221) 50 % glycerol solution (25 mg/2.5 mL) approx. 550 U/mg 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 ₂ O	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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH (cm²/μmol)

*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)

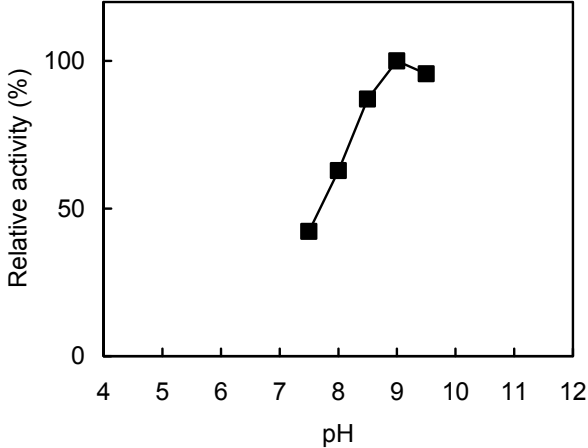


Fig. 1 pH profile

■ Tris-HCl

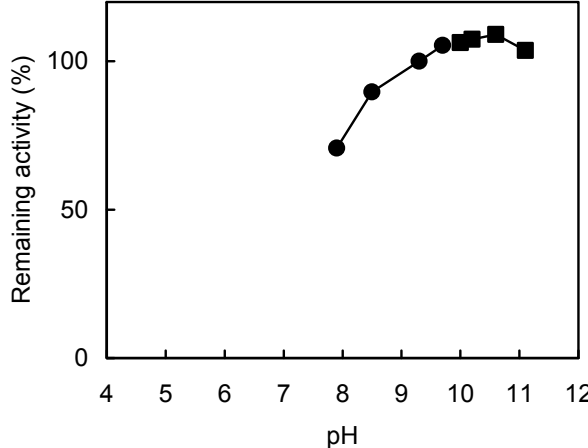


Fig. 2 pH stability

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

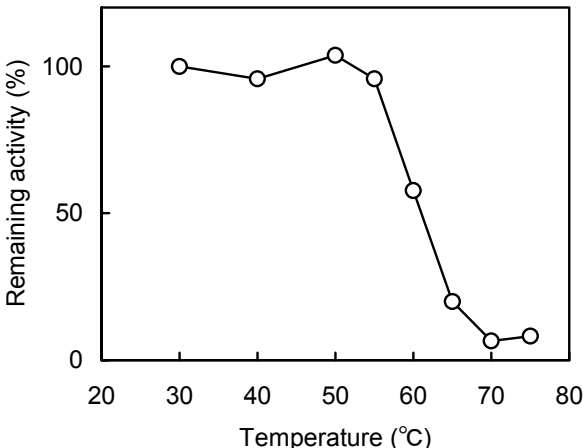


Fig. 3 Thermal stability

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

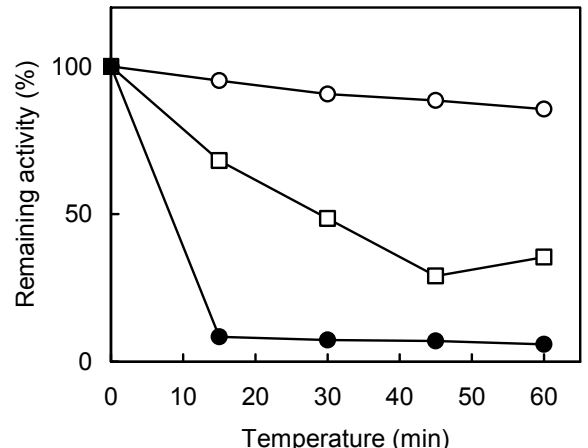


Fig. 4 Thermal stability

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

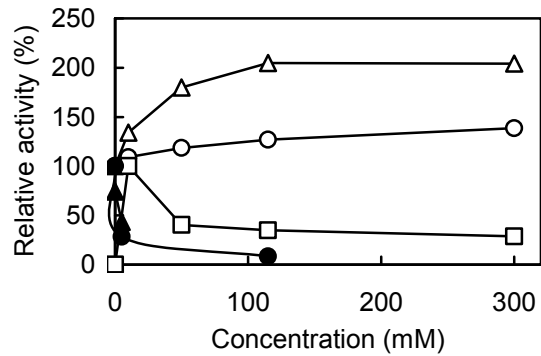


Fig. 5 Effect of various cations on the activity of Polynucleotide phosphorylase in the following Assay Method

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, □ MgCl₂, ● CaCl₂, ▲ ZnCl₂

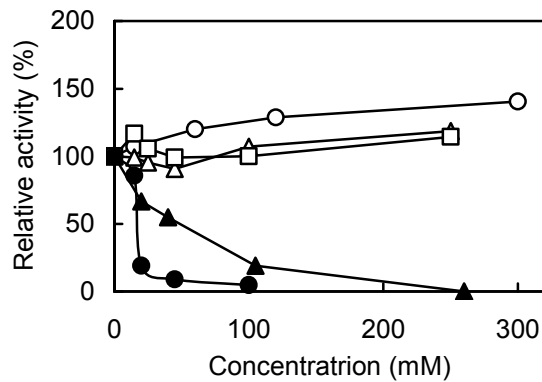
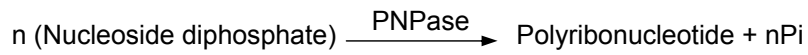


Fig. 6 Effect of various anions on the activity of Polynucleotide phosphorylase in the following Assay Method

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, △ CH₃COONa, □ Na₂SO₄,
● NaHCO₃, ▲ NaH₂PO₄

FOR POLYMERIZATION REACTION



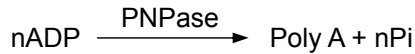
PROPERTIES

Specific activity	: more than 2,000 U/mg protein	
Contaminants	: (as PNPase activity = 100 %)	
	Ribonuclease	< 0.0001 %
Optimum pH	: 9.0 - 9.5	
Michaelis constants	: (79 mM Tris-HCl buffer, pH 9.5, at 60 °C)	
	ADP	2.2 mM
Substrate specificity (25 mM)	: (125 mM Tris-HCl buffer, pH 9.0, at 37°C)	
	ADP	100 %
	CDP	41 %
	IDP	117 %

ASSAY

Principle

The radioactivity of Poly A that obtained by polymerizing [C^{14}] ADP is measured according to the following reaction.



Unit Definition

One unit of activity is defined as the amount of PNPase that polymerizes 1 μmol of ADP at 60 °C per hour.

Solutions

- I Buffer solution ; 0.2M Tris-HCl, pH 9.5
- II ADP solution ; 100mM (0.507 g ADP disodium salt·2H₂O/(9.0 mL distilled water + 1.0 mL 1 N-NaOH))
- III [C^{14}] ADP solution ; (0.05 mCi/2.5 mL, 43.3 mCi/mmol; New England Nuclear)
- IV Mixed ADP solution ; (mix 9.5 volume of solution II and 5 volume of solution III)
- V EDTA solution ; 10 mM (0.037 g EDTA disodium salt·2H₂O/10 mL distilled water)
- VI MgCl₂ solution ; 1 M (2.033 g MgCl₂·6H₂O/10 mL distilled water)

Preparation of Enzyme Solution

referred to "Assay based on depolymerizing poly A"

Procedure

- Prepare the following reaction mixture and pipette 150 μL of the reaction mixture into a test tube.

Solution I	590 μL	Solution VI	80 μL
Solution IV	150 μL	H ₂ O	580 μL
Solution V	100 μL		
- Preincubate at 60 °C for about 5 minutes.
- Start the reaction by adding 10 μL of the enzyme solution.
- After exactly 4 minutes, stop the reaction by adding 0.3 mL of 10 % trichloroacetic acid (TCA) solution and mixing. Allow to stand for about 10 minutes.
- Filter the solution obtained in Procedure 4 through Whatman GF/C. Wash twice inside of the test tube with 5 mL of 1% TCA solution and filter the solution through the same filtering paper. Further, wash surface of the filter funnel with 5 mL of 1% TCA solution.
- Air-dry the filter paper at room temperature.
- Measure the counts of radioactivity with liquid scintillator. Prepare a calibration curve of dpm vs. ADP concentration previously.

Calculation

$$\text{Volume activity (U/mL)} = \text{dpm (measured)} \times A \times \frac{1000}{10} \times \frac{60}{4} \times \text{d.f.}$$

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

A ; ADP concentration (μmol) per dpm

d.f. ; dilution factor

*Protein concentration ; determined by the absorbance at 280nm (Abs280), where 1 Abs280 = 1 mg/mL

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

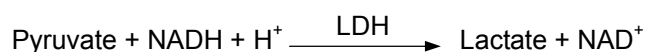
APPLICATION

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.



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·2H₂O/(9.0 mL distilled water + 1.0 mL 1 N NaOH))
- III NADH solution ; 13.1 mM (0.100 g NADH disodium salt·3H₂O/10 mL distilled water)
- IV Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
- V MgCl₂ solution ; 1.0 M (20.33 g MgCl₂·6H₂O/100 mL distilled water)
- VI KCl solution ; 2.5 M (18.64 g KCl/100 mL distilled water)
- VII Lactate dehydrogenase (LDH) ; (from hog muscle, Roche Diagnostics K.K., No. 127 221) 50 % glycerol solution (10 mg/mL) approx. 550 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	22.71 mL	Solution V	0.48 mL
Solution II	2.40 mL	Solution VI	0.90 mL
Solution III	0.45 mL	Solution VII	0.06 mL
Solution IV	3.00 mL		
2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

Calculation

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH (cm²/μmol)

*Protein concentration ; determined by Bradford's method

REFERENCE

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

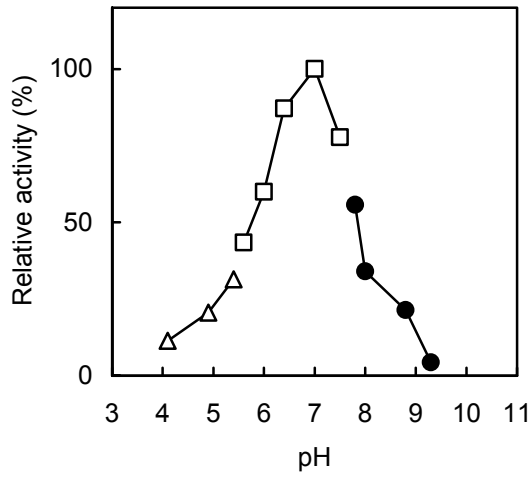


Fig. 1 pH profile

(Δ acetate, \square imidazole-HCl, \bullet Tris-HCl)

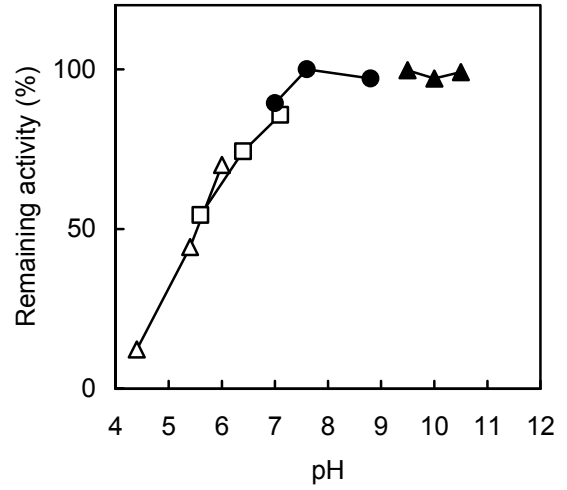


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the following buffer solution (0.1 M);
 Δ acetate, \square imidazole-HCl, \bullet Tris-HCl, \blacktriangle carbonate)

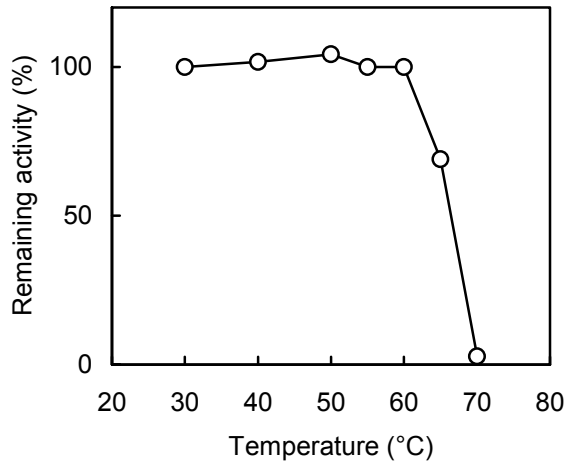


Fig. 3 Thermal stability

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

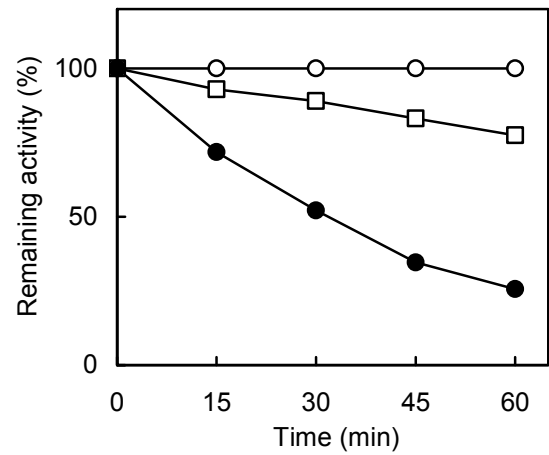


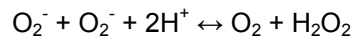
Fig. 4 Thermal stability

(treated in 0.1 M Tris-HCl buffer, pH 8.5
 \circ 55 °C, \square 60 °C, \bullet 65 °C)

SUPEROXIDE DISMUTASE (SOD)

[EC 1.15.1.1]

from *Bacillus stearothermophilus*



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

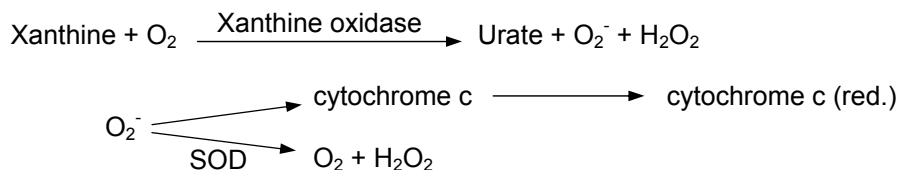
APPLICATION

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.



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·2H₂O/50 mL distilled water)
- V Xanthine oxidase (XOD) ; (from buttermilk, Sigma-Aldrich Co., No. X-1875) suspension in 2.3 M (NH₄)₂SO₄ 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 ($\Delta\text{Abs}\cdot\text{test}$)*.
5. Add 0.005 mL of Solution I in place of enzyme solution and measure the same above 4 ($\Delta\text{Abs}\cdot\text{blank}$).

*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

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

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

d.f. ; dilution factor

*Protein concentration ; determined by Bradford's method

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1. Bridgen, J., Harris, J.I., and Kolb, E.; *J. Mol. Biol.*, **105**, 333 (1976)
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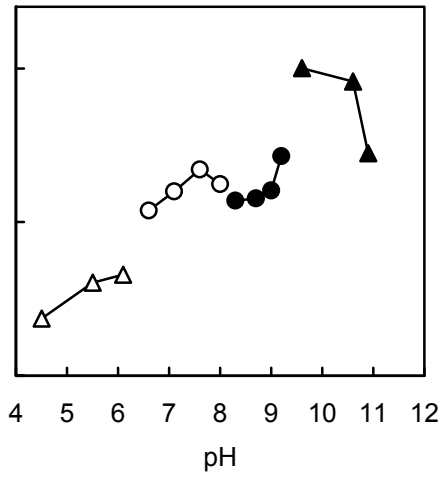


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle carbonate)

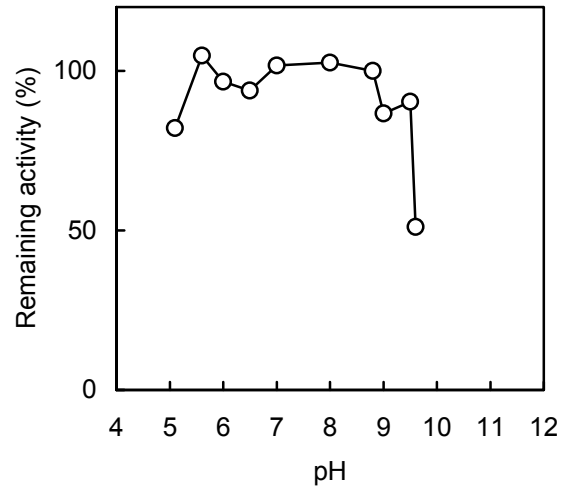


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the
Britton-Robinson buffer)

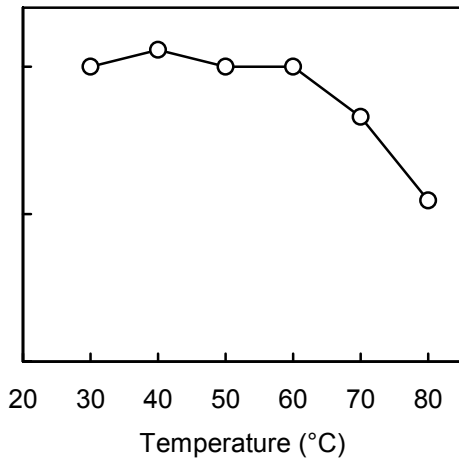


Fig. 3 Thermal stability

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

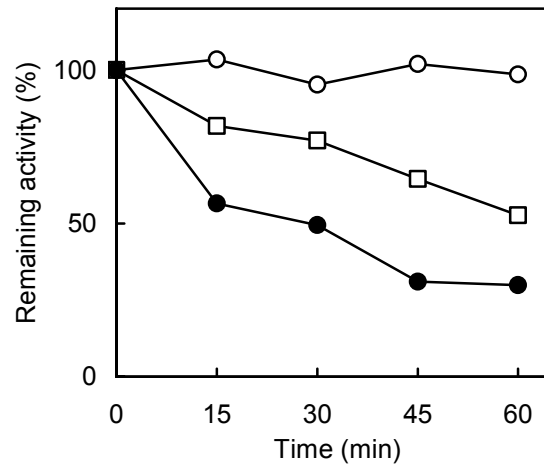


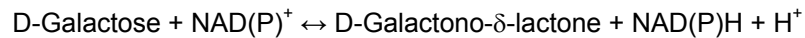
Fig. 4 Thermal stability

(treated in 0.1 M potassium
phosphate buffer, pH 7.5
 \circ 60 °C, \square 70 °C, \bullet 80 °C)

GALACTOSE DEHYDROGENASE (GalDH)

[EC 1. 1. 1. 48]

from recombinant *E. coli*



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

Stable at 4 to 10 °C for at least eight months

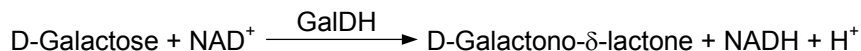
APPLICATION

This enzyme is useful for determination of galactose.

ASSAY

Principle

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



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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by the Bradford's method

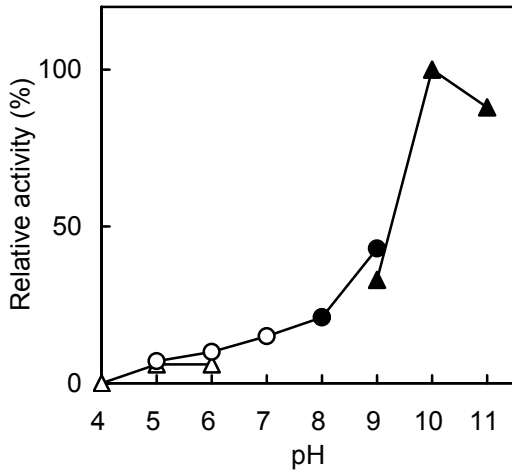


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle Glycine-KOH)

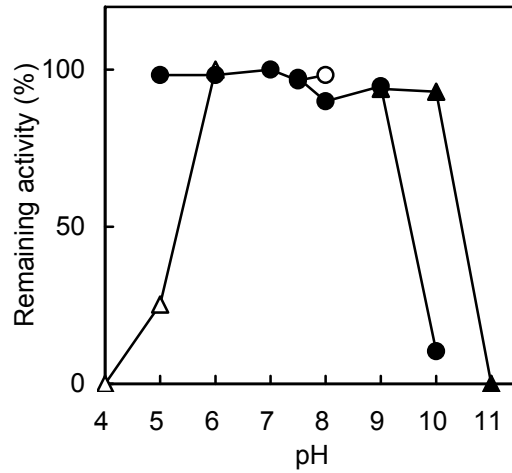


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the
following buffer solution (0.1 M);
 Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle Glycine-KOH)

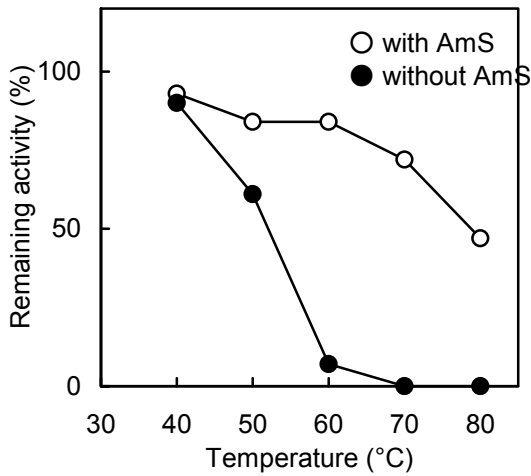


Fig. 3 Thermal stability

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

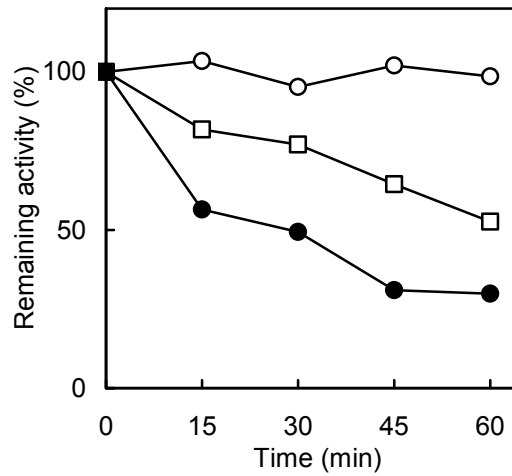


Fig. 4 Thermal stability

(treated in 25 mM potassium
phosphate buffer pH 7.5 at
 \circ 40 °C, \square 50 °C, \bullet 60 °C
without ammonium sulphate.)

GLUCOSE DEHYDROGENASE (GlcDH2)

[EC 1. 1. 1. 47]

from recombinant *E. coli*



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.



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	17.26mL
Solution II	0.50mL
Solution III	2.00mL
Solution IV	0.24mL
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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by the absorbance at 280nm (Abs_{280}), where 1 Abs_{280} = 1 mg/mL

REFERENCE

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

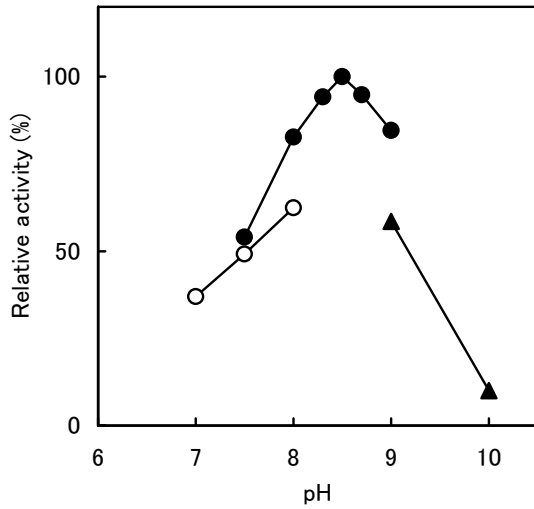


Fig. 1 pH profile

[○ phosphate, ● Tris-HCl, ▲ glycine]

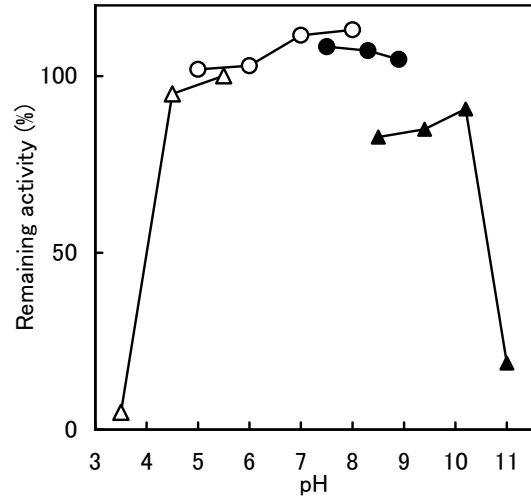


Fig. 2 pH stability

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

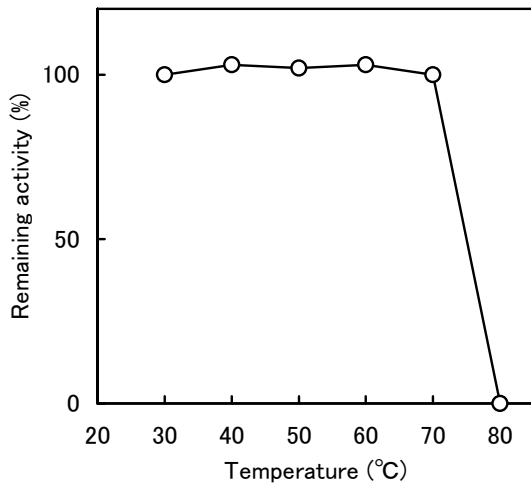


Fig. 3 Thermal stability

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

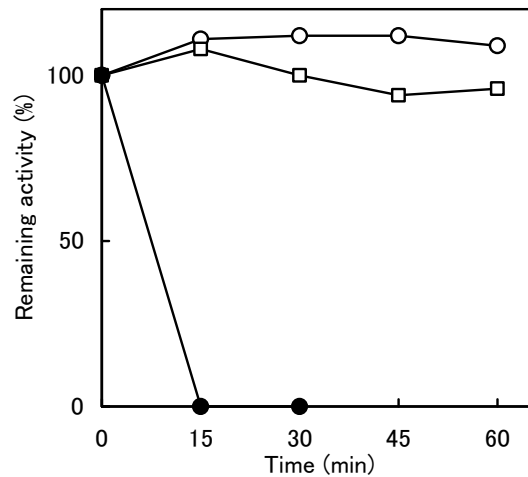


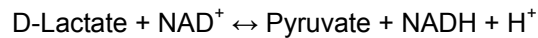
Fig. 4 Thermal stability

(treated for in 0.1M phosphate buffer, pH 6.5, containing 3M NaCl and 0.1% BSA, ○ 60°C, □ 70°C, ● 80°C)

D-LACTATE DEHYDROGENASE (D-LDH)

[EC 1. 1. 1. 28]

from *Microorganism*



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⁺	

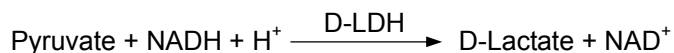
STORAGE

Stable at -20 °C at least one year

ASSAY

Principle

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



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-3H₂O/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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

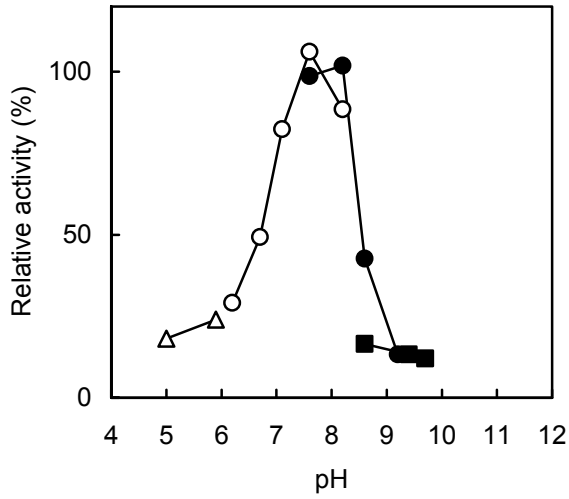


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
● Tris-HCl, ■ Gly-KOH)

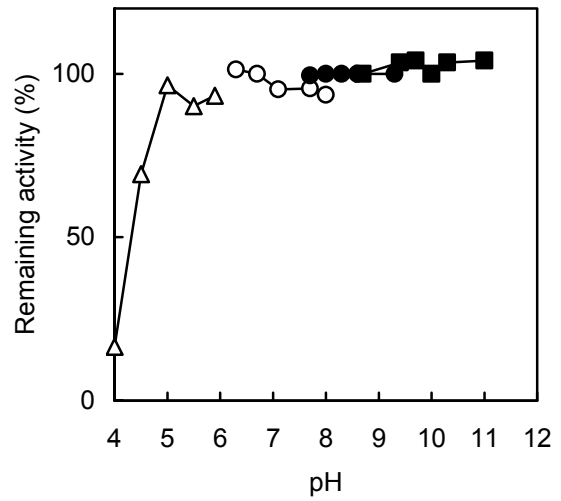


Fig. 2 pH stability

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

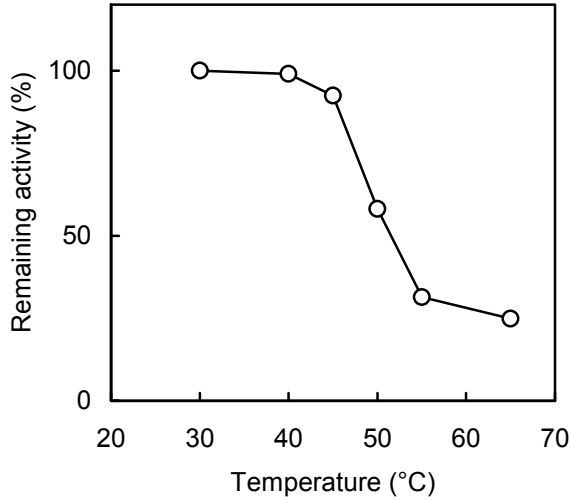


Fig. 3 Thermal stability

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

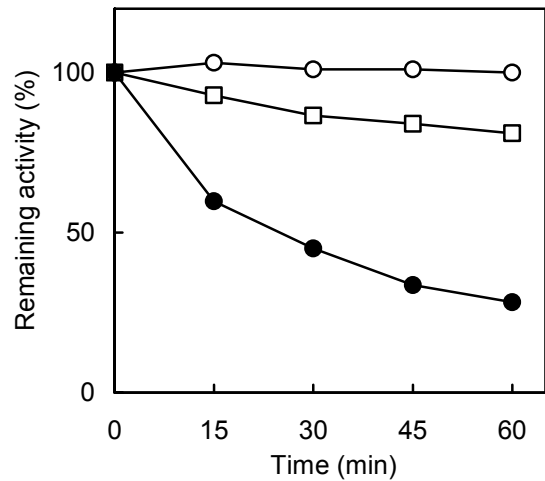


Fig. 4 Thermal stability

(treated in 0.1 M potassium phosphate buffer, pH 7.0
 \circ 40 °C, \square 45 °C, ● 50 °C)

MALATE DEHYDROGENASE (MDH)

[EC 1. 1. 1. 37]

from *Microorganism*



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-HCl 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

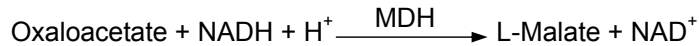
APPLICATION

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.



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-3H₂O/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 ₂ O	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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

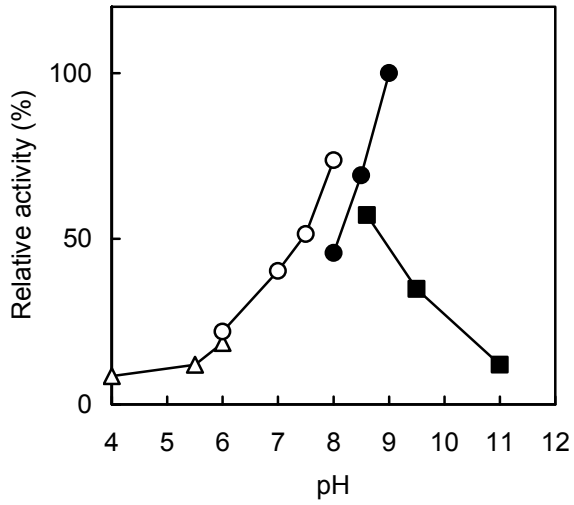


Fig. 1 pH profile

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

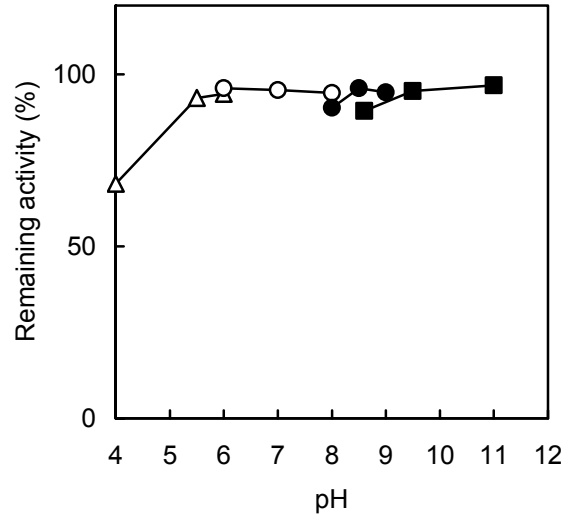


Fig. 2 pH stability

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

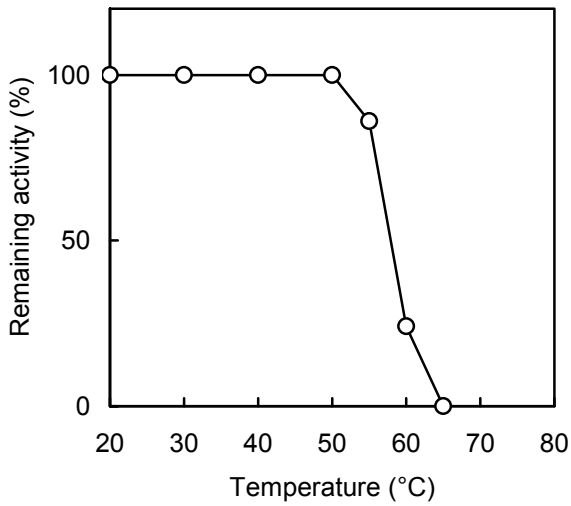


Fig. 3 Thermal stability

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

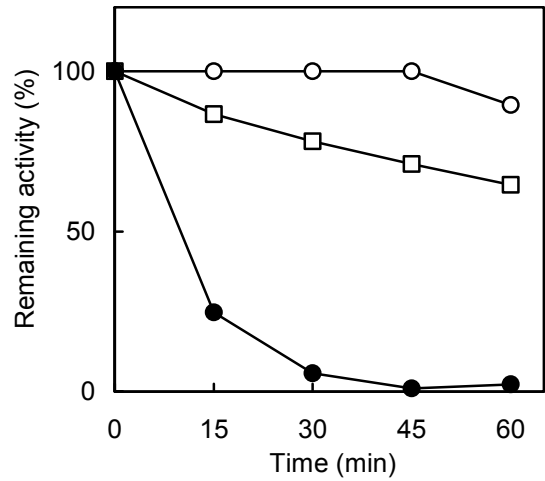


Fig. 4 Thermal stability

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

MUTAROTASE (MRO)

[EC 5. 1. 3. 3]

from *Microorganism*

α -D-glucose \leftrightarrow 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

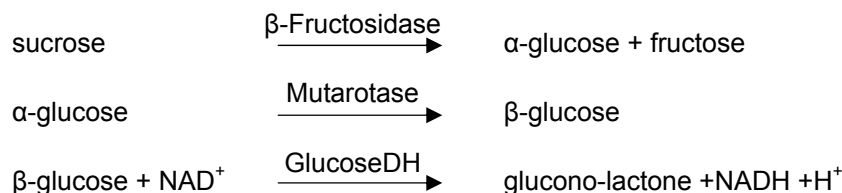
APPLICATION

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.



Unit Definition

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

Solutions

- I HEPES buffer ; 50 mM (1.19 g HEPES / 100mL distilled water, adjust pH to 7.5 with NaOH)
- II Sucrose solution ; 16.7 mM (60 mg Sucrose / 10 mL distilled water)
- III NAD⁺ solution ; 120 mM (851 mg NAD-2Na / 10 mL distilled water)
- IV Glucose dehydrogenase solution ; > 6 u/mL (GDH (e.g. Unitika M1G221) / HEPES buffer (Soln. I))
- V β -Fructosidase solution ; 30 ku/mL (β -Fructosidase (e.g. Sigma 14504) / 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 diluent (20 mM potassium phosphate pH7.3 containing 1mg/mL BSA).

Procedure

1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
 - Solution I 20.00mL
 - Solution II 1.00mL
 - Solution III 0.50mL
2. Add 0.02 mL of the Solution IV and 0.02 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 (Δ Abs1) in the linear portion of curve.
6. Run the procedure 1 to 5 with the enzyme diluent instead of the enzyme solution (Δ Abs2).

Calculation

$$\text{Volume activity (U/mL)} = \frac{(\Delta\text{Abs1} - \Delta\text{Abs2}) \times (3.00 + 0.10)}{6.22 \times 0.02 \times 10} \times \text{d.f.}$$

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH (cm²/ μ mol)

10 ; conversion factor

*Protein concentration ; determined by the absorbance at 280nm (Abs280),
where 1 Abs280 = 1 mg/mL

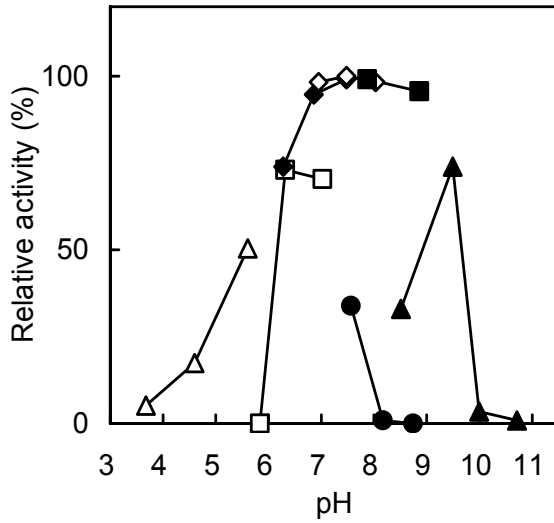


Fig. 1 pH profile

(Δ acetate, \square MES, \blacklozenge PIPES,
 \diamond HEPES, \bullet Tris-HCl, \blacksquare Bicine,
 \blacktriangle Glycine-KOH)

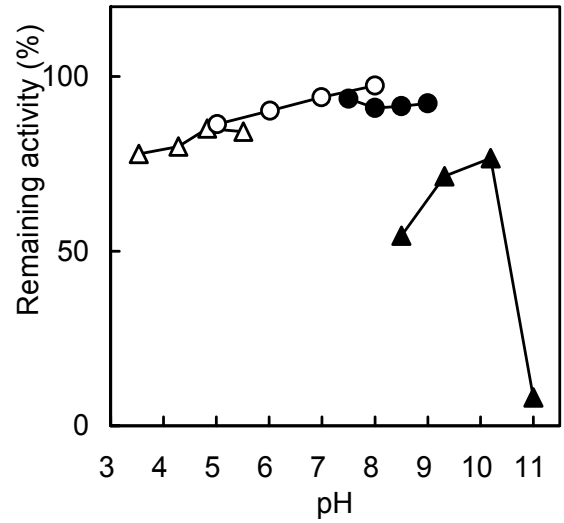


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the
following buffer solution (0.1 M)
containing 0.1 % BSA;
 Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle Glycine-KOH)

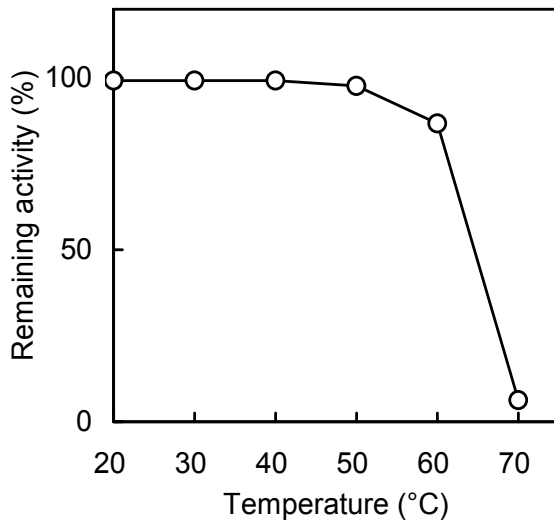


Fig. 3 Thermal stability

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

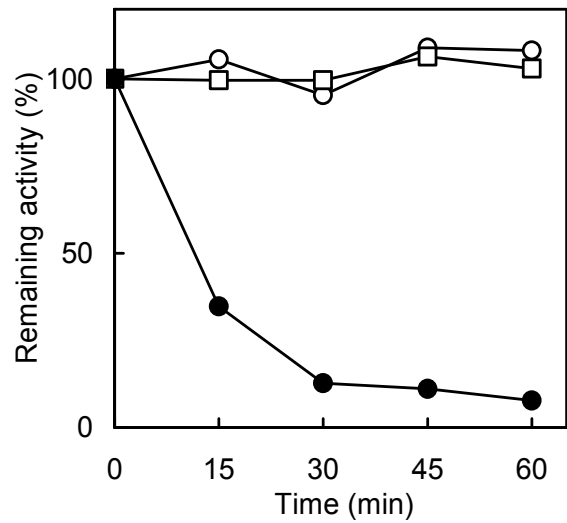


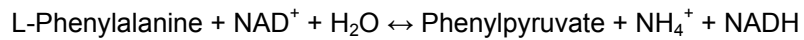
Fig. 4 Thermal stability

(treated in 0.1 M potassium phosphate
buffer pH 6.5, 0.1 % BSA at \circ 40 °C,
 \square 50 °C, \bullet 60 °C.)

PHENYLALANINE DEHYDROGENASE (PheDH)

[EC 1.4.1.20]

from *Thermoactinomyces intermedius*



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

ASSAY

Principle

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



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 ₂ O	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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*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)

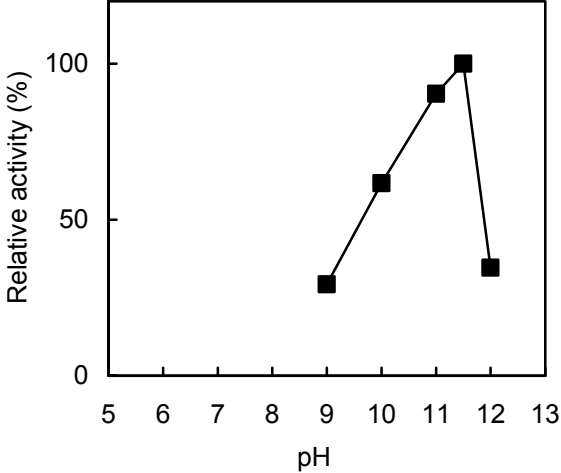


Fig. 1 pH profile

■ Gly-KCl-KOH

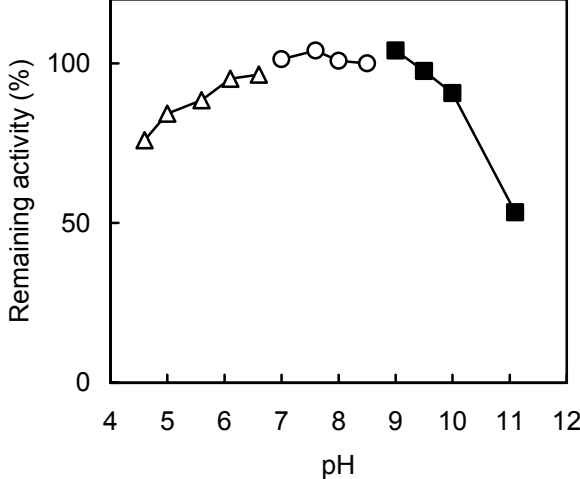


Fig. 2 pH stability

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

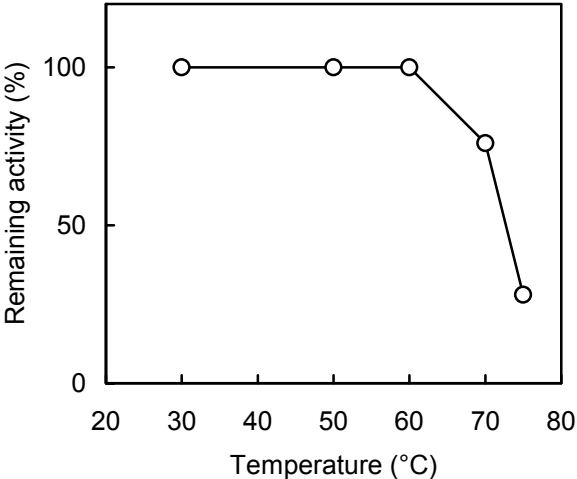


Fig. 3 Thermal stability

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

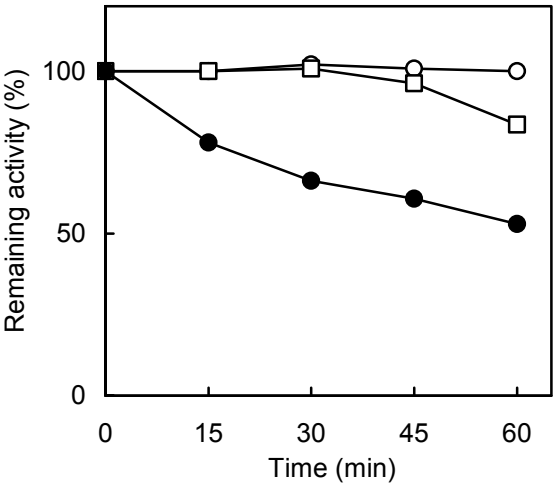


Fig. 4 Thermal stability

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

6-PHOSPHOGLUCONATE DEHYDROGENASE (DECARBOXYLATING) (6PGDH)

[EC 1. 1. 1. 44]

from *Microorganism*



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 Glycylglycine 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	

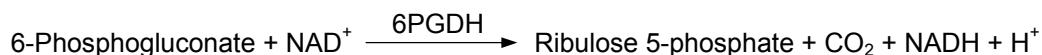
STORAGE

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.



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·2H₂O/10 mL distilled water)
- III NAD⁺ solution ; 50 mM (0.332 g NAD⁺ free acid/10 mL distilled water)
- IV MgCl₂ solution ; 1 M (20.33 g MgCl₂·6H₂O/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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

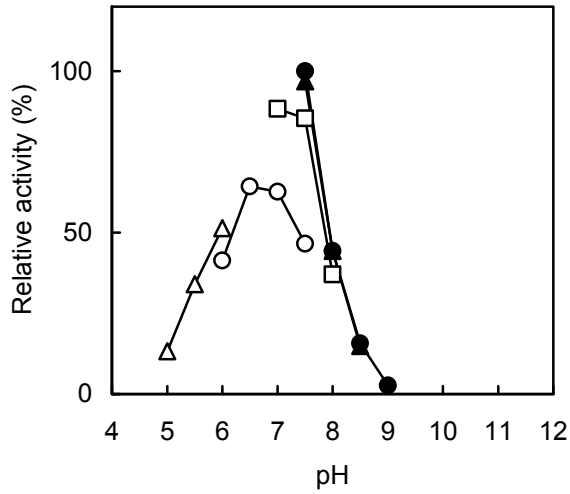


Fig. 1 pH profile

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

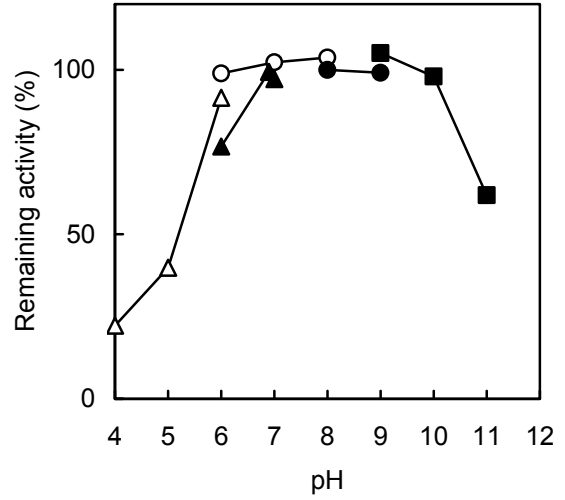


Fig. 2 pH stability

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

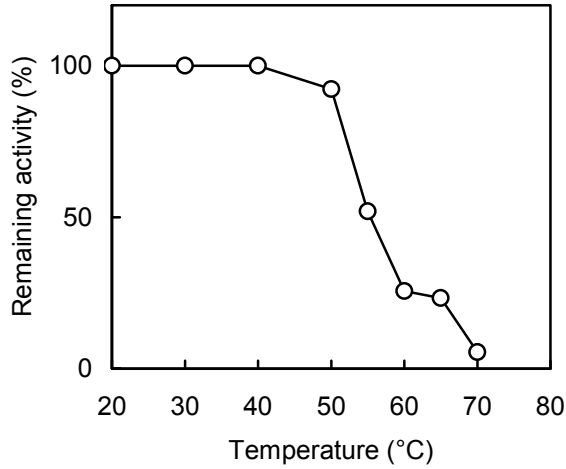


Fig. 3 Thermal stability

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

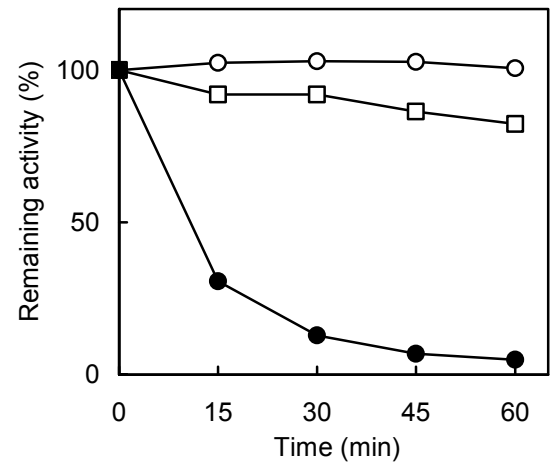


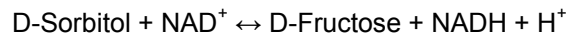
Fig. 4 Thermal stability

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

SORBITOL DEHYDROGENASE (SorDH)

[EC 1.1.1.14]

from *Microorganism*



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

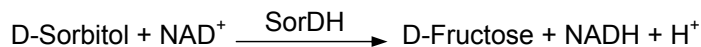
APPLICATION

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.



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

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

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

d.f. ; dilution factor

6.22 ; millimolar extinction coefficient of NADH ($\text{cm}^2/\mu\text{mol}$)

*Protein concentration ; determined by Bradford's method

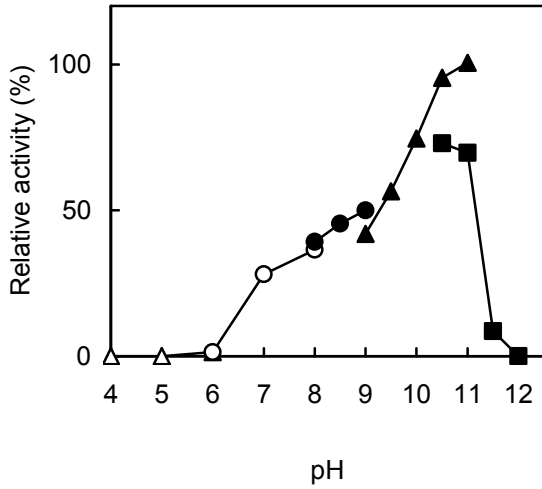


Fig. 1 pH profile

(Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle Gly-KOH,
 \blacksquare Na₂HPO₄-NaOH)

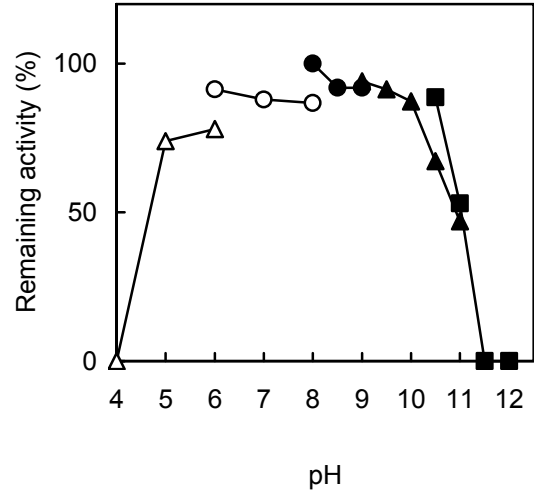


Fig. 2 pH stability

(treated for 24 hr at 4 °C in the
 following buffer solution (0.1 M);
 Δ acetate, \circ phosphate,
 \bullet Tris-HCl, \blacktriangle Gly-KOH,
 \blacksquare Na₂HPO₄-NaOH)

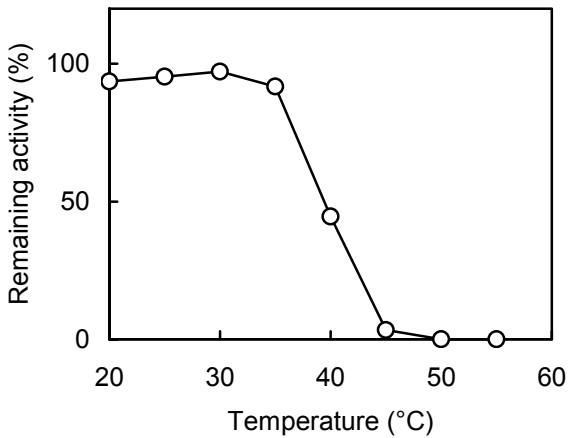


Fig. 3 Thermal stability

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

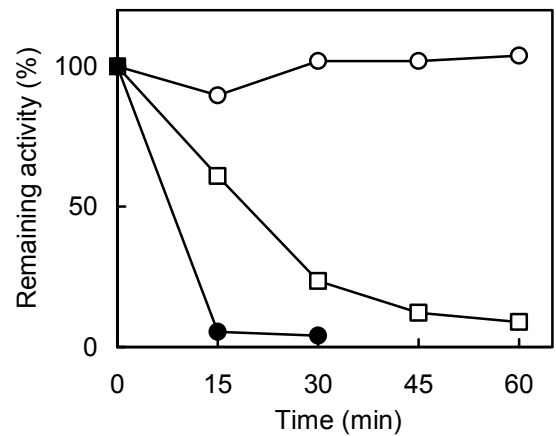


Fig. 4 Thermal stability

(treated in 0.1 M Tricine -NaOH
 buffer, pH 8.0
 \circ 35 °C, \square 40 °C, \bullet 45 °C)