ENZYMES IVD

ENZYMES

THERMOSTABLE

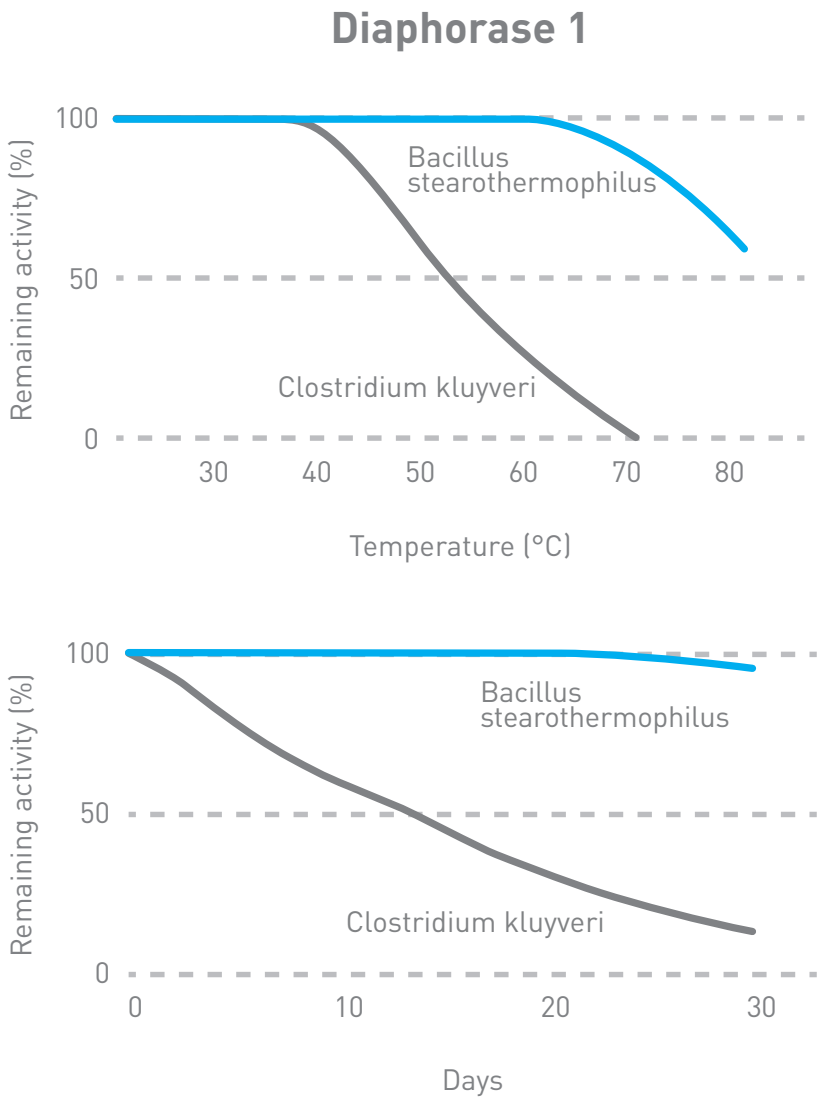
NIPRO

INDEX

NIPRO ENZYMES

Nipro develops enzymes that maintain all positive catalytic characteristics but limit the disadvantages. As a consequence Nipro enzymes gain the following attributes:

- Our enzymes are **stable for a longer time** and in a **wider temperature** range due to the isolation out of thermophilic bacteria.
- Our **enzymes have a high purity** and subsequently a **high degree of reliability** because of our unique culturing process.



These characteristics make our enzymes suitable for very demanding applications such as clinical diagnostics, the synthesis of pharmaceutical intermediates, food analysis,...

BENEFITS FOR YOU

The thermophilic properties combined with the high purity and activity degree of our enzymes ensure:

FOR OUR CUSTOMERS:

- The possibility of storage in a wider temperature range
- Lower requirement of enzyme volume
- Less waste production
- Optimal kit reliability

FOR END USERS:

- Fewer calibrations of equipment
- Reliable test results
- The possibility of storage in a wider temperature range

Acetate Kinase

ATP + Acetate ⟷ ADP + Acetylphosphate

AK

EC 2. 7. 2. 1

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.

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

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM Imidazole-HCl, pH 7.2
2. ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N NaOH))
3. Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
4. NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)
5. $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$ /100 mL distilled water)
6. KCl solution ; 2.5 M (18.64 g KCl/100 mL distilled water)
7. Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C
8. Lactate dehydrogenase (LDH) ; (from pig heart, Oriental Yeast Co. Ltd., Product Code: LDH-02) ammonium sulfate suspension, approx. 5,000 U/mL at 25 °C
9. Sodium acetate solution ; 2 M (27.22 g sodium acetate·$3H_2O$/100 mL distilled water)

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	16.92 mL	Solution 3:	1.80 mL	Solution 5:	0.60 mL	Solution 7:	0.12 mL
Solution 2:	3.00 mL	Solution 4:	0.60 mL	Solution 6:	0.90 mL	Solution 8:	0.06 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.60 mL of Solution 9 and 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

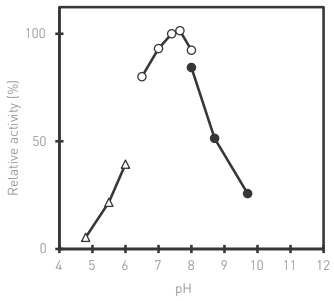


Fig. 1 pH profile

△ acetate, ○ phosphate,
● Tris-HCl

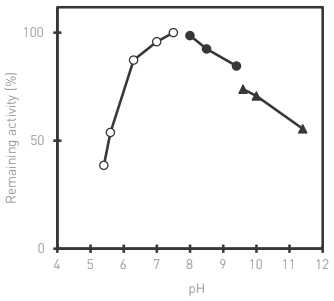


Fig. 2 pH stability

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

○ phosphate,
● Tris-HCl, ▲ carbonate

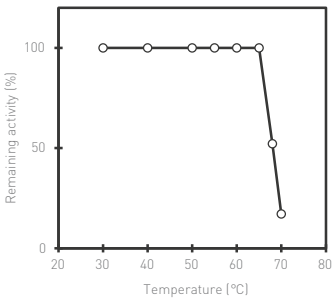


Fig. 3 Thermal stability

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

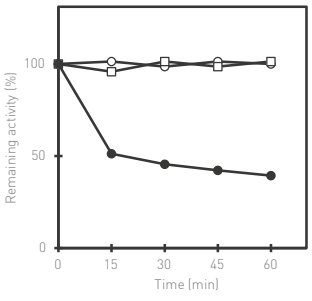


Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.5

○ 60 °C, □ 65 °C, ● 70 °C

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)

Adenylate Kinase

ATP + AMP ⟷ 2 ADP

AdK

EC 2. 7. 4. 3

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.

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

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM Imidazole-HCl, pH 6.5
2. AMP solution ; 50 mM (0.250 g AMP disodium salt·$6H_2O$/10 mL distilled water)
3. ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N NaOH))
4. NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$ /10 mL distilled water)
5. Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
6. $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
7. KCl solution ; 2.5 M (18.64 g KCl/100 mL distilled water)
8. Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C
9. Lactate dehydrogenase (LDH) ; (from pig heart, Oriental Yeast Co. Ltd., Product Code: LDH-02) ammonium sulfate suspension, approx. 5,000 U/mL at 25 °C

PREPARATION OF ENZYME SOLUTION

Dissolve the lyophilized enzyme with distilled water and dilute to 2.5 to 5 U/mL with 50 mM Tris-HCl buffer, pH 8.5.

PROCEDURE

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

Solution 1:	26.70 mL	Solution4:	0.60 mL	Solution 7:	1.20 mL
Solution 2:	0.24 mL	Solution 5:	0.18 mL	Solution 8:	0.09 mL
Solution 3:	0.30 mL	Solution 6:	0.60 mL	Solution 9:	0.09 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

d.f.: dilution factor
2: according to the reaction that forms 2 µmol of ADP, one unit of activity of Adk is defined to form 2 µmol of ADP.
6.22: millimolar extinction coefficient of NADH (cm^2/µmol)
*Protein concentration ; determined by Bradford's method

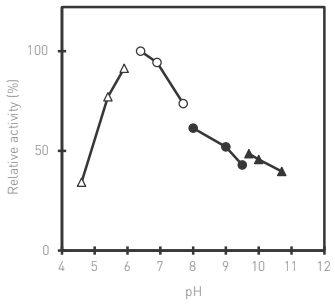


Fig. 1 pH profile

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

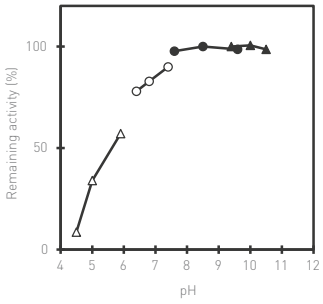


Fig. 2 pH stability

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

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

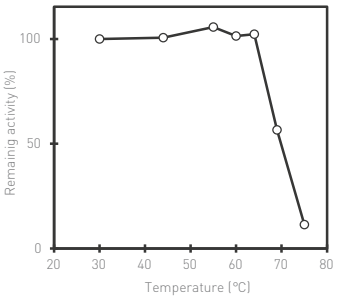


Fig. 3 Thermal stability

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

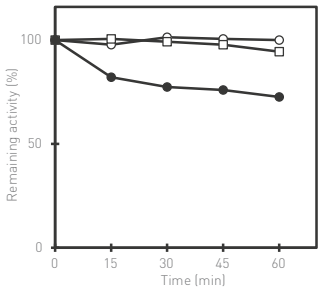


Fig. 4 Thermal stability

treated in 0.1M Tris-HCl buffer, pH 9.0

○ 60 °C, □ 65 °C, ● 70 °C

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

Alanine Dehydrogenase

L-Alanine + NAD^+ + H_2O ⟷ Pyruvate + NH_4^+ + NADH

AlaDH

EC 1. 4. 1. 1

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.

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 250 mM Glycine-NaOH, pH 10.5
2. 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)
3. 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 1:	15.00 mL	Solution 3:	1.50 mL
Solution 2:	10.00 mL	H_2O:	3.50 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

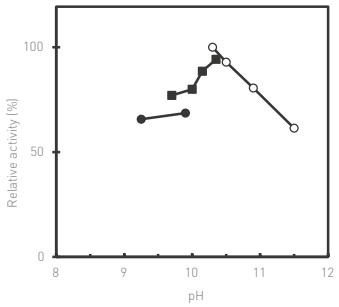


Fig. 1 pH profile
● Tris-HCl, ■ Gly-KOH,
○ phosphate,

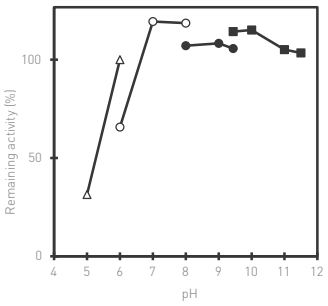


Fig. 2 pH stability
treated for 24 hr at 4 °C in the following buffer solution (0.1 M)
△ acetate, ○ phosphate,
● Tris-HCl, ■ Gly-KOH

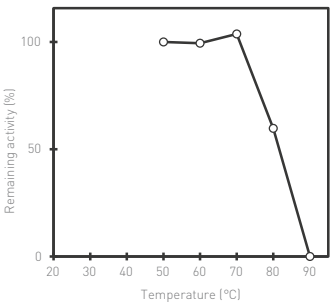


Fig. 3 Thermal stability
treated for 15 min in 0.1 M Gly-KOH buffer, pH 9.0

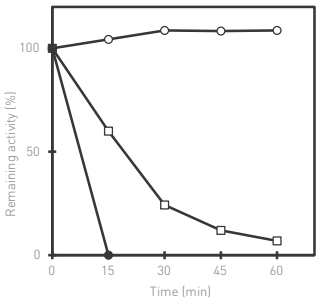


Fig. 4 Thermal stability
treated in 0.1 M Gly-KOH buffer, pH 9.0
○ 70 °C, □ 80 °C, ● 90 °C

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

Alanine Racemase

D-Alanine ↔ L-Alanine

AlaR

EC 5. 1. 1. 1

Bacillus stearothermophilus

SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at least one year at -25 °C.

ASSAY

PRINCIPLE

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

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

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

UNIT DEFINITION

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

SOLUTIONS

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

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	16.50 mL	Solution 3:	0.75 mL	H_2O:	8.25 mL
Solution 2:	3.00 mL	Solution 4:	1.50 mL		

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

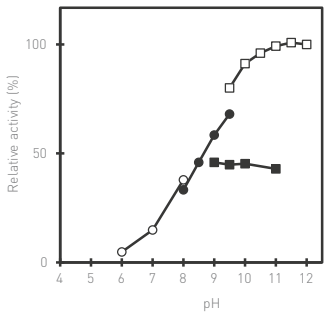


Fig. 1 pH profile

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

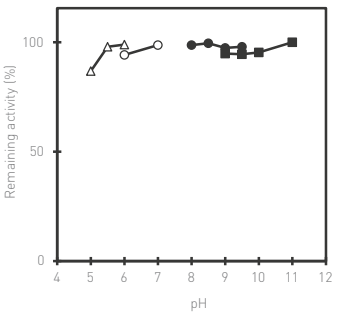


Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.2 M)

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

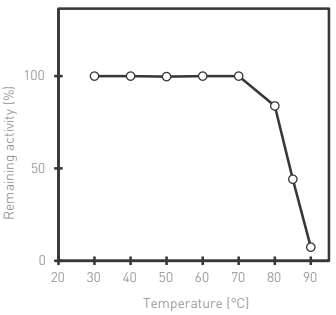


Fig. 3 Thermal stability

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

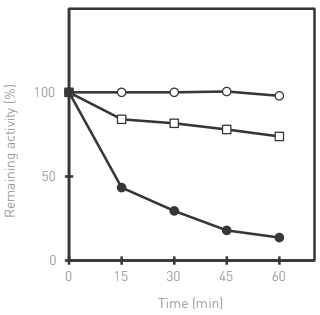


Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 9.0

○ 70 °C, □ 80 °C, ● 85 °C

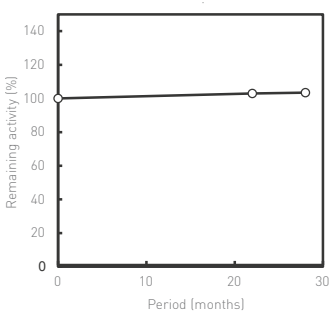


Fig. 5 Stability (Liquid form) at -25 °C

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

Alcohol Dehydrogenase

Alcohol + NAD^+ ⟷ Aldehyde + NADH + H^+

ZM-ADH

EC 1 .1 .1 .1

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.

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 80 mM Glycine-KOH, pH 9.5.
2. NAD+ solution ; 10 mM (0.0663 g NAD+ free acid/10 mL distilled water).
3. 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 1 mg/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 1:	22.90 mL
Solution 2:	6.00 mL
Solution 3:	1.10 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

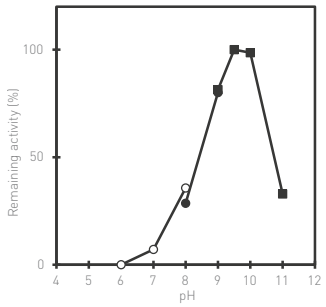


Fig. 1 pH profile

○ phosphate, ● Tris-HCl, ■ Gly-KOH

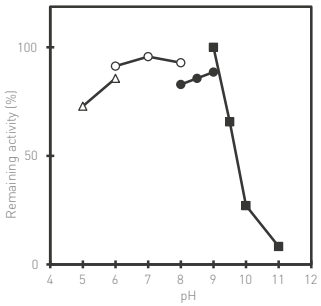


Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M), containing 0.5 mM $CoCl_2$;

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

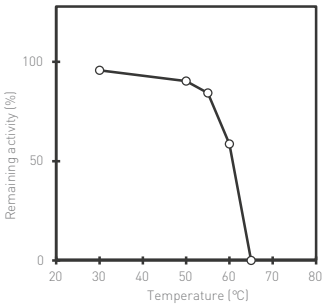


Fig. 3 Thermal stability

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

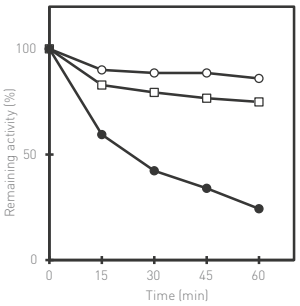


Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer containing 0.2 mM CoCl2, pH 6.5

○ 50 °C, □ 55 °C, ● 60 °C

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

Bilirubin Oxidase

2 Bilirubin + O_2 ⟶ 2 Biliverdin + 2 H_2O

BOD3

EC 1.3.3.5

Trachyderma tsunodae

SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at -20 °C for one year

APPLICATION

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

ASSAY

PRINCIPLE

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

Phenol + ½O_2 + H_2O $\xrightarrow{BOD3}$ Quinone and/or Phenoxy radical + H_2O_2

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 300 mM Potassium phosphate buffer, pH 7.0
2. 4-Aminoantipyrine (4-AA) solution ; 24.6 mM (0.25 g 4-AA / 50 mL distilled water)
3. Phenol solution ; 420 mM (1.98 g phenol/50mL distilled water)
4. Peroxidase*1 (POD) solution ; 240 U/mL (2,400 U/10mL distilled water)

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	4.00 mL	Solution 4:	0.40 mL
Solution 2:	0.40 mL	H_2O:	6.40 mL
Solution 3:	0.40 mL		

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

*1 POD:TOYOBO Co., LTD. Grade 3 #PEO-302

CALCULATION

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

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

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

TABLE 1 SUBSTRATE SPECIFICITY OF BOD3

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

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

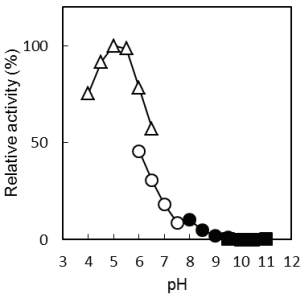


Fig. 1 pH profile

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

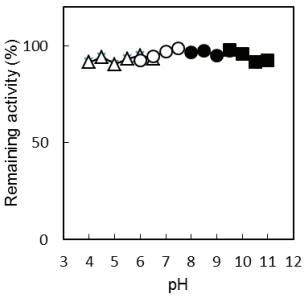


Fig. 2 pH stability
treated for 24 hr at 4 °C in the following buffer solution (50 mM)

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

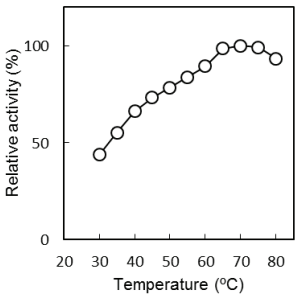


Fig. 3 Thermal stability

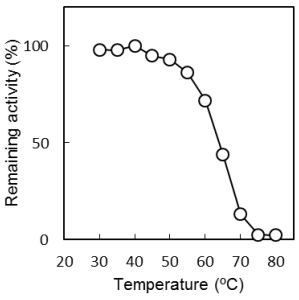


Fig. 4 Thermal stability
treated for 15 min in 20 mM potassium phosphate buffer, pH 7.0

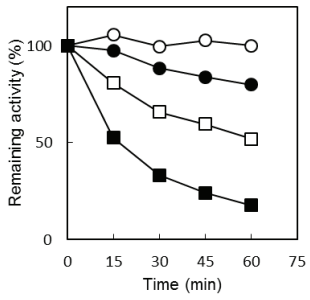


Fig. 5 Thermal stability
treated in 20 mM potassium phosphate buffer, pH 7.0

○ 50°C, ● 55°C, □ 60°C, ■ 65°C

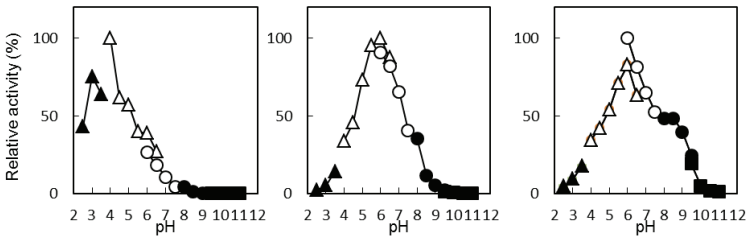


Fig. 6 pH profile (ABTS*3)

Fig. 7 pH profile (Bilirubin C*4)

Fig. 8 pH profile (Bilirubin F*4)

Measured in 20 mM buffer
▲ Glycine-HCL, △ acetate, ○ phosphate, ● Tris-HCL, ■ Glycine-KOH

*3 2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic Acid) Diammonium Salt

*4 Bilirubin C (conjugated type) and Bilirubin F (free type) are from [Interference check. APlus] (Sysmex, Kobe, Japan)

Diaphorase 3

NAD(P)H + Acceptor(ox.) + H^+ ⟷ NAD(P)$^+$ + Acceptor(red.)

Di-3

EC 1. 6. 99. -

Recombinant E. coli

SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at -20 to 5 °C for one year

APPLICATION

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

ASSAY

PRINCIPLE

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 500 mM Tris-HCl, pH8.5
2. NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)
3. 2,6-Dichlorophenolindophenol (DCIP) solution ; 1.2 mM (2.0 mg DCIP sodium salt·$2H_2O$/5mL distilled water) (prepare freshly)

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

1. Prepare the following reaction mixture and pipette 2.28 mL of reaction mixture and 0.12 mL of Solution 3 into a cuvette.

Solution 1:	3.00 mL	H_2O:	23.22 mL
Solution 2:	2.28 mL		

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

CALCULATION

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

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

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

TABLE 1. SUBSTRATE SPECIFICITY OF DIAPHORASE

Acceptor	DCIP*1		NTB*2		MTT*3	
$Km^{Acceptor}$ (mM)	0.02		0.15		0.9	
Km^{NADH} (mM)	0.37		0.01		0.05	
Km^{NADPH} (mM)	32.7		0.31		2.0	
Optimum pH	8.0		> 10		8.0	
Assay Mixture	Tris-HCl (pH 8.5) NAD(P)H DCIP	50 mM 1 mM 0.06 mM	TEA (pH 7) NAD(P)H NBT Triton X-100	50 mM 1 mM 0.5 mM 0.1 %	TEA (pH 7.5) NAD(P)H MTT Triton X-100	50 mM 1 mM 0.5 mM 0.5 %
Wavelength for measurement (nm)	600		550		565	
Extinction coefficient ($cm^2/\mu mol$)	19		12.4		20	

EFFECT OF BSA ON THE ACTIVITY OF DIAPHORASE: (SEE BELOW)

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 BELOW)

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.

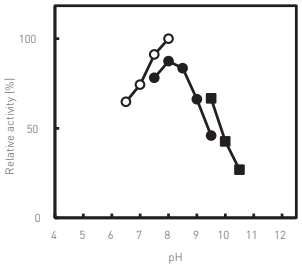


Fig. 1 pH profile

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

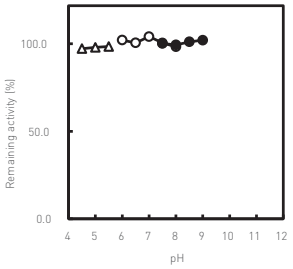


Fig. 2 pH stability

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

△ acetate, ○ phosphate,
● Tris-HCl

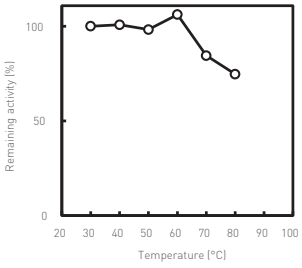


Fig. 3 Thermal stability

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

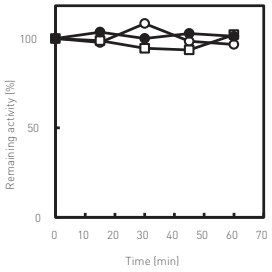


Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.5

○ 50 °C, □ 60 °C, ● 70 °C

PH PROFILES OF DI-3 (ACCEPTOR; NTB OR MTT)

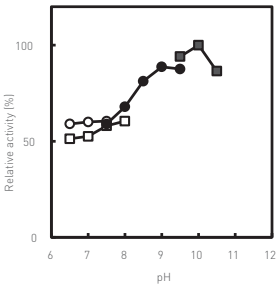


Fig. 5 pH profile (NTB)

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

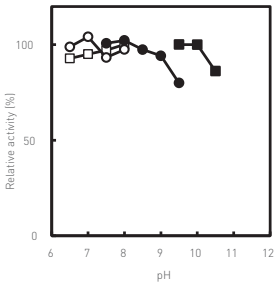


Fig. 5 pH profile ((MTT)

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

REFERENCE : 1. Mains, I., Power, D.M., Thomas, E.W. and Buswell J. A.; Biochem. J., 191, 457 (1980)
*1 2,6-Dichlorophenolindophenol
*2 Nitrotetrazolium Blue
*3 Thiazolyl Blue Tetrazolium Bromide

Diaphorase 22

NADH + Acceptor(ox.) + H^+ ⟷ NAD^+ + Acceptor(red.)

Di-22

EC 1. 8. 1. 4

Recombinant E.coli

SPECIFICATION

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

PROPERTIES

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

STORAGE

Store at -20 °C

APPLICATION

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

ASSAY

PRINCIPLE

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM HEPES, pH 7.0
2. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution ; 10 mM (20 mg MTT disodium salt·$2H_2O$/5 mL distilled water)
3. NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$ /10 mL distilled water)
4. Triton solution ; 10 % (1 mL Triton X-100 dilute with distilled water up to 10 mL)

* (Table 1), next page

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	15.00 mL	Solution 4:	1.50 mL
Solution 2:	1.50 mL	H_2O:	10.80 mL
Solution 3:	1.20 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 565 nm per minute (ΔAbs_{565}) in the linear portion of curve.

CALCULATION

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

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

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

TABLE 1. SUBSTRATE SPECIFICITY OF DIAPHORASE 22

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

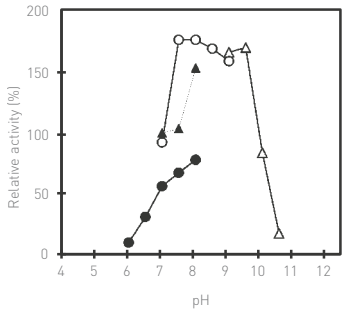


Fig. 1 pH profile

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

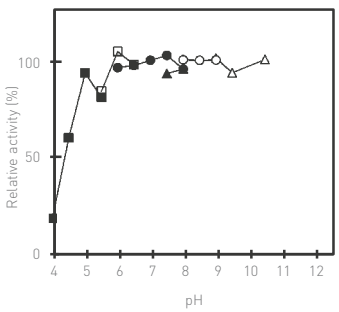


Fig. 2 pH stability
treated for 24 hr at 4 °C in the following buffer Solution (0.1 M)

△ Gly-KOH, ○ Bicine
● phosphate, ▲ HEPES,
□ MES., ■ Citrate

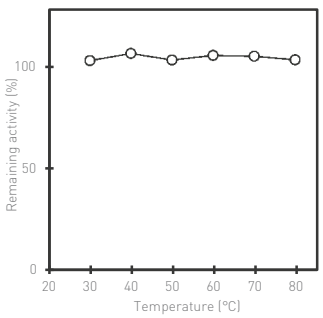


Fig. 3 Thermal stability
treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

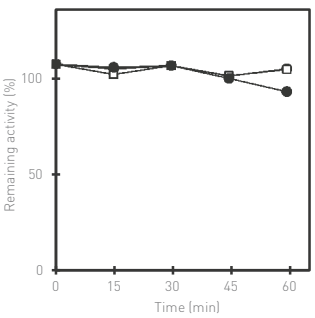


Fig. 4 Thermal stability
treated for in 0.1 M potassium phosphate buffer, pH 7.5

○ 60 °C, □ 70 °C, ● 80 °C

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

D-Lactate Dehydrogenase

D-Lactate + NAD^+ ⟷ Pyruvate + NADH + H^+

D-LDH

EC 1. 1. 1. 28

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	:	(NH4)2 SO4, 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.

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

UNIT DEFINITION

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

SOLUTIONS

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

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	28.00 mL	Solution 3:	0.80 mL
Solution 2:	1.20 mL		

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

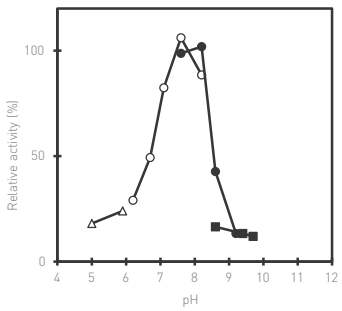


Fig. 1 pH profile

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

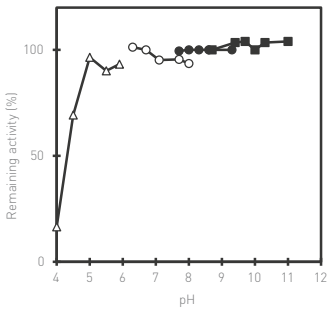


Fig. 2 pH stability

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

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

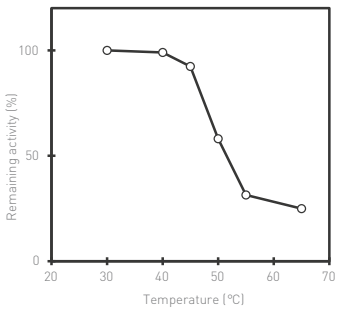


Fig. 3 Thermal stability

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

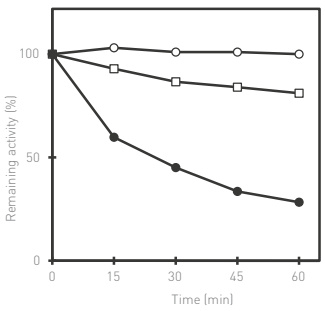


Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer, pH 7.0

○ 40 °C, □ 45 °C, ● 50 °C

Galactose Dehydrogenase

D-Galactose + NAD(P)$^+$ ⟷ D-Galactono-δ-lactone + NAD(P)H + H$^+$

GalDH

EC 1. 1. 1. 48

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

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

APPLICATION

This enzyme is useful for determination of galactose.

ASSAY

PRINCIPLE

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM Tris-HCl, pH9.1 (at 30°C)
2. NAD$^+$ solution ; 100 mM
3. D-Galactose solution ; 1 M
4. 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 1:	27.60 mL	Solution 3:	1.50 mL
Solution 2:	0.90 mL		

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

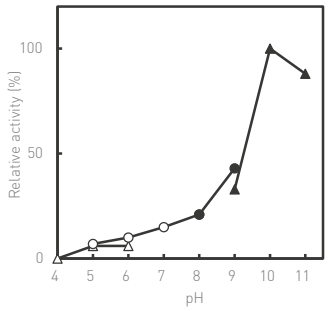


Fig. 1 pH profile

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

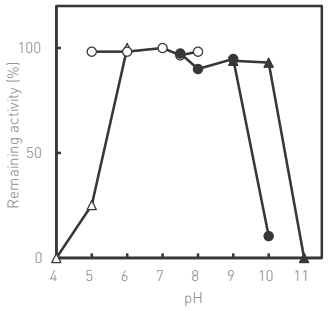


Fig. 2 pH stability

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

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

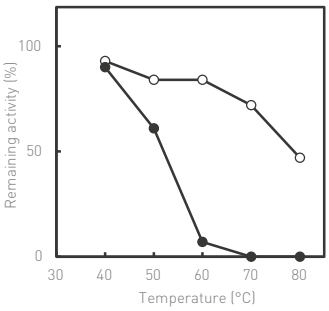


Fig. 3 Thermal stability

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

○ with AmS, ● without AmS

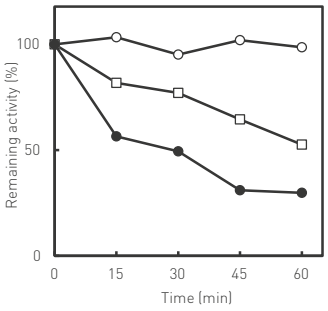


Fig. 4 Thermal stability

treated in 25 mM potassium phosphate buffer pH 7.5 without ammonium sulphate at

○ 40 °C, □ 50 °C, ● 60 °C

Glucokinase

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

ZM-GlcK

EC 2. 7. 1. 2

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	:	(60 mM 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.

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

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

UNIT DEFINITION

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

SOLUTIONS

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

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 1:	20.07 mL	Solution 4:	0.60 mL
Solution 2:	1.50 mL	Solution 5:	7.50 mL
Solution 3:	0.30 mL	Solution 6:	0.03 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

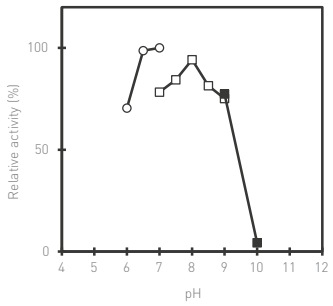


Fig. 1 pH profile
○ MES-KOH, □ TEA-NaOH, ■ Gly-KOH

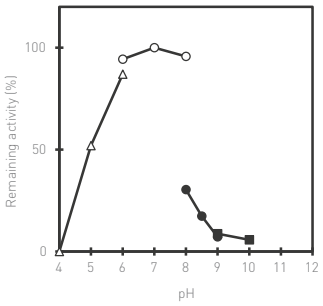


Fig. 2 pH stability
treated for 24 hr at 4 °C in the following buffer solution (0.1 M)
Δ acetate, ○ phosphate, ● Tris-HCl, ■ Gly-KOH

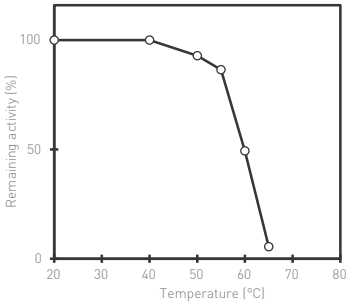


Fig. 3 Thermal stability
treated for 15 min in 0.1 M phosphate buffer, pH 7.0

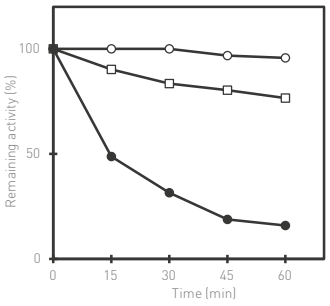


Fig. 4 Thermal stability
treated in 0.1 M phosphate buffer , pH 7.0
○ 40 °C, □ 50 °C, ● 60 °C

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

Glucokinase

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

GlcK

EC 2. 7. 1. 2

Bacillus stearothermophilus

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 and 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.

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM Tris-HCl, pH 9.0
2. ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N NaOH))
3. $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
4. $NADP^+$ solution ; 22.5 mM (0.188 g $NADP^+$ sodium salt·$4H_2O$)/10 mL distilled water)
5. Glucose solution ; 40 mM (0.072 g glucose (anhyd.)/10 mL distilled water)
6. Glucose-6-phosphate dehydrogenase (G6PDH) ; (from yeast. Roche Diagnostics K.K., No. 127 671) suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/2 mL) approx. 140 U/mg at 25 °C

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	17.97 mL	Solution 4:	1.20 mL
Solution 2:	1.20 mL	Solution 5:	9.00 mL
Solution 3:	0.60 mL	Solution 6:	0.03 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

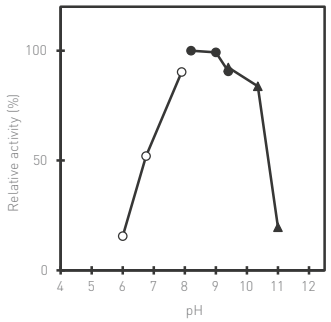


Fig. 1 pH profile

○ phosphate, ● Tris-HCl, ▲ carbonate

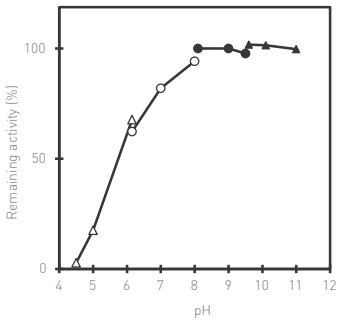


Fig. 2 pH stability

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

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

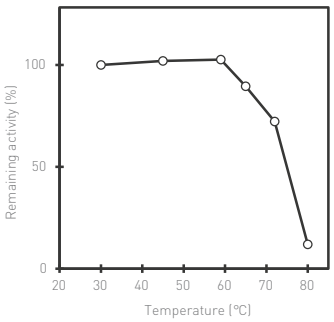


Fig. 3 Thermal stability

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

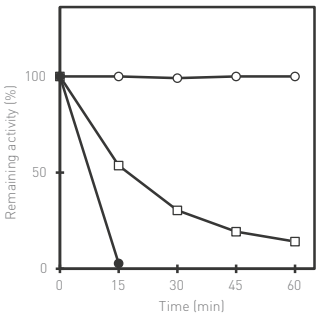


Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 8.9

○ 60 °C, □ 70 °C, ● 80 °C

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)

Glucokinase 2

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

GlcK2

EC 2. 7. 1. 2

Recombinant E.coli

SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at -20°C for at least one year

APPLICATION

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

ASSAY

PRINCIPLE

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

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM Tris-HCl, pH 9.0
2. ATP solution ; 100 mM (0.605 g ATP disodium salt·$3H_2O$/(8.2 mL distilled water + 1.8 mL 1 N-NaOH))
3. $MgCl_2$ solution ; 1 M (20.33 g $MgCl_2 \cdot 6H_2O$/100 mL distilled water)
4. $NADP^+$ solution ; 22.5 mM [(0.172 g $NADP^+$ monosodium salt or 0.177 g NADP+ disodium salt)/10 mL distilled water]
5. Glucose solution ; 40 mM (0.072 g glucose (anhyd.)/10 mL distilled water)
6. 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 1:	17.97 mL	Solution 4:	1.20 mL
Solution 2:	1.20 mL	Solution 5:	9.00 mL
Solution 3:	0.60 mL	Solution 6:	0.03 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

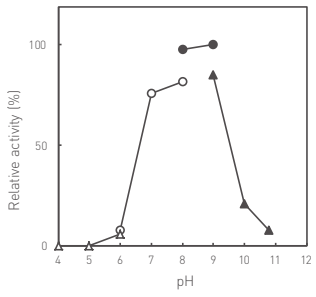


Fig. 1 pH profile

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

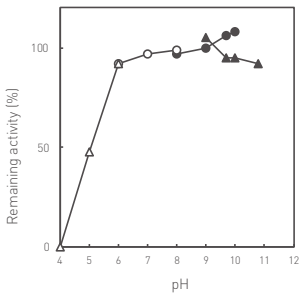


Fig. 2 pH stability

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

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

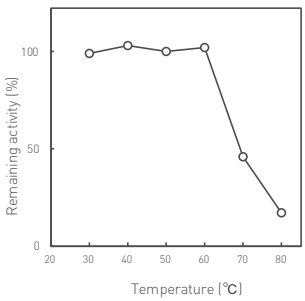


Fig. 3 Thermal stability

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

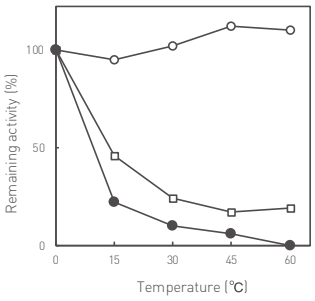


Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 8.9

○ 60 °C, □ 70 °C, ● 80 °C

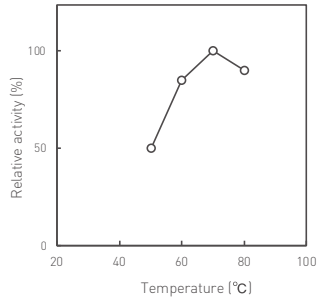


Fig. 5 Thermal activity

defined as 100% at 70 °C

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)

Glucose Dehydrogenase

D-Glucose + $NAD(P)^+$ ⟷ D-Glucono-δ-lactone + $NAD(P)H + H^+$

GlcDH2

EC 1. 1. 1. 47

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.

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM Tris-HCl, pH 8.5 (at 25 °C)
2. NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)
3. D-Glucose solution ; 1 M (1.802 g glucose (anhyd.)/10 mL distilled water)
4. 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 1 mg/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 1:	17.26 mL	Solution 3:	2.00 mL
Solution 2:	0.50 mL	Solution 4:	0.24 mL

2. Incubate at 37 °C for about 3 minutes.
3. Add 0.015 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

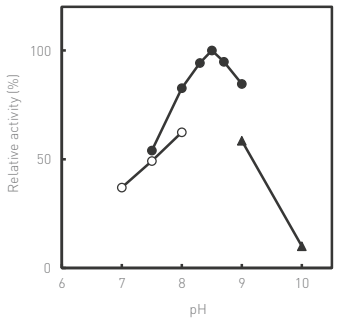


Fig. 1 pH profile

○ phosphate, ● Tris-HCl, ▲ glycine

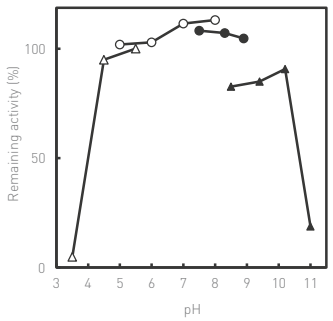


Fig. 2 pH stability

treated for 24 hr at 4 °C in the following buffer solution (0.1 M) containg 3 M NaCl

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

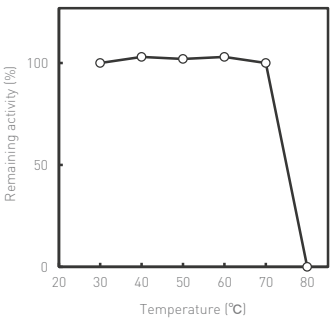


Fig. 3 Thermal stability

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

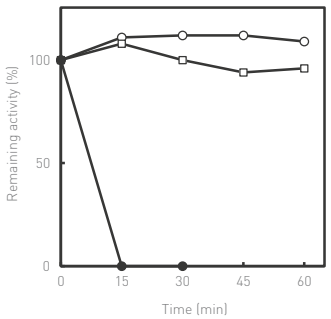


Fig. 4 Thermal stability

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

○ 60 °C, □ 70 °C, ● 80 °C

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

Glucose-6-Phosphate Dehydrogenase

ZM-G6PDH

EC 1. 1. 1. 49

Zymomonas mobilis

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

SPECIFICATION

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

PROPERTIES

Molecular weight	:	ca. 208,000
Subunit molecular weight	:	ca. 52,000
Optimum pH	:	8.0 ..... (Fig. 1)
pH stability	:	5.0 - 10.0 ..... (Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 50 °C. ..... (Fig. 3, 4)
Michaelis constants	:	(30 mM 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.

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 50 mM Tris-HCl, pH 8.0
2. NAD$^+$ solution ; 100 mM (0.663 g NAD$^+$ free acid/10 mL distilled water)
3. Glucose-6-phosphate (G6P) solution ; 33 mM (0.112 g G6P disodium salt $2H_2O$/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 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 1:	26.40 mL
Solution 2:	0.90 mL
Solution 3:	2.70 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

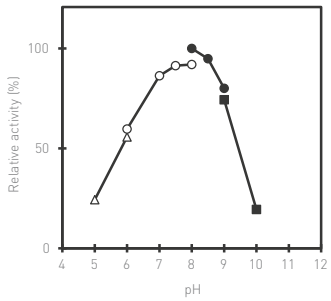


Fig. 1 pH profile

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

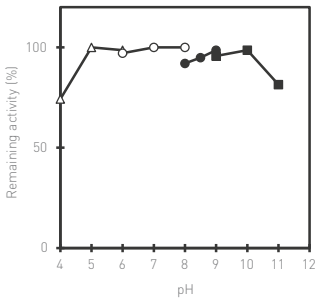


Fig. 2 pH stability

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

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

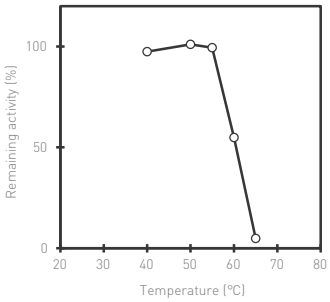


Fig. 3 Thermal stability

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

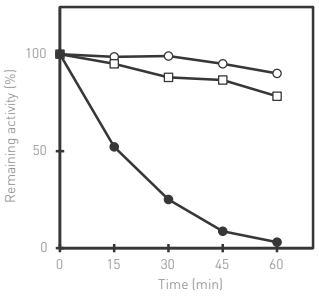


Fig. 4 Thermal stability

treated in 0.1 M phosphate buffer, pH 7.0

○ 50 °C, □ 55 °C, ● 60 °C

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

Glucose-6-Phosphate Dehydrogenase 2 (Recombinant)

G6PDH2

EC 1. 1. 1. 49

Recombinant E. coli

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

SPECIFICATION

State	:	Lyophilized
Specific activity	:	more than 400 U/mg protein
Contaminants	:	(as G6PDH activity = 100 %)
		Glucokinase ..... < 0.01 %
		Phosphoglucomutase ..... < 0.02 %
		6-Phosphogluconate dehydrogenase ..... < 0.01 %
		Glutathione reductase ..... < 0.01 %

PROPERTIES

Subunit molecular weight	:	ca. 54,000
Optimum pH	:	8.1 ..... (Fig. 1)
pH stability	:	3.5-10.5 ..... (Fig. 2)
Thermal stability	:	No detectable decrease in activity up to 45 °C. ..... (Fig. 3, 4)
Michaelis constants	:	(100 mM Tris-HCl buffer, pH 7.8, at 30 °C)
		Glucose 6-phosphate ..... 0.15 mM
		NAD+ ..... 0.41 mM
		NADP+ ..... 0.008 mM
Substrate specificity	:	Glucose 6-phosphate ..... 100 %
		Ribose 5-phosphate ..... 0 %
		Mannose 6-phosphate ..... 0 %
		Fructose 6-phosphate ..... 4.3 %
		Fructose 1,6-bisphosphate ..... 0 %
		Glucose 1-phosphate ..... 0 %
		Glucosamine 6-phosphate ..... 0.1 %
		6-Phosphogluconate ..... 0 %
	:	NAD+ ..... 100 %
		NADP+ ..... 73 %
Effecters	:	..... (Fig. 5, 6)

STORAGE

Store at -20 °C

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.

Glucose 6-phosphate + NAD$^+$ $\xrightarrow{\text{G6PD2}}$ Gluconolactone 6-phosphate + NADH + H$^+$

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 100 mM Tris-HCl, pH 7.8
2. MgCl2 solution ; 90 mM
3. NAD+ solution ; 100 mM
4. Glucose 6-phosphate (G6P) solution ; 100 mM

PREPARATION OF ENZYME SOLUTION

Dissolve the lyophilized enzyme with distilled water and dilute to approx.5 U/ml with 20 mM Tris-HCl buffer, pH 7.5 containing 0.1% bovine serum albumin.

PROCEDURE

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

Solution 1:	15.00 ml	Solution 3:	1.00 ml	Distilled water:	12.40ml
Solution 2:	1.00 ml	Solution 4:	0.60 ml		

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

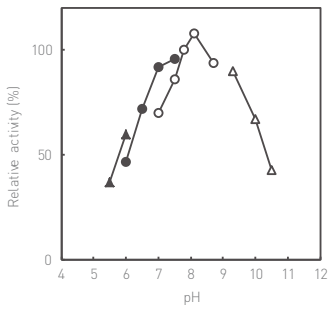


Fig. 1 pH profile

△ Gly-KOH, ○ Tris-HCl
● phosphate, ▲ MES

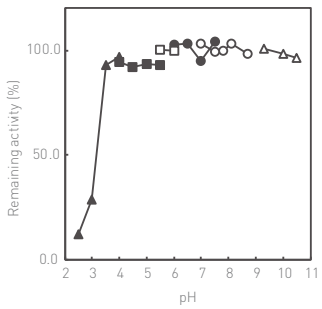


Fig. 2 pH stability

Treated for 24 hr at 4°C in the following buffer solution (0.1 M), :

△ Gly-KOH, ○ Tris-HCl, ● phosphate,
▲ Gly-HCl, □ MES, ■ Citrate

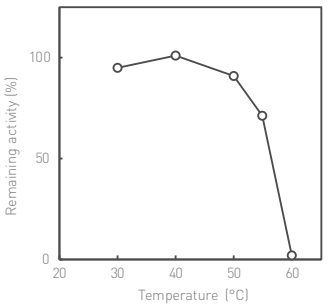


Fig. 3 Thermal stability

For 15 min in 20mM Tris-HCl buffer, pH 7.5

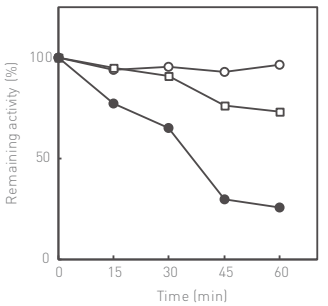


Fig. 4 Thermal stability

Treated for in 20mM Tris-HCl buffer, pH 7.5

○ 45 °C, □ 50 °C, ● 55 °C

TABLE 1 EFFECT OF CHEMICALS

Enzymes are diluted with 50 mM Tris-HCl bufferpH 7.5 includeing each chemicals and incubated 1hourat 30°C. Afterincubation, the remaining activity of G6PDH2 was measured.

Chemical	Final Conc.	Remaining activily
None (D.W.)	-	100%
$CaCl_2$	2 mM	91%
$FeCl_3$	2 mM	23%
$FeSO_4$	2 mM	0%
$MgCl_2$	2 mM	98%
$MnCl_2$	2 mM	96%
KF	2 mM	103%
NaF	20mM	85%
EDTA	5 mM	97%
$(NH_4)_2SO_4$	20mM	101%
Urea	2 mM	106%
Na-cholate	1%	108%
Triton-X100	1%	99%
Brij 35	1%	95%
SDS	0.1%	0%
Tween 20	0.1%	105%
Span 20	0.1%	103%
DAC	0.1%	1%

Glycerol-3-Phosphate Dehydrogenase

G3PDH

EC 1.1.1.8

Recombinant E. coli

Glycerol-3-phosphate + NAD^+ ⟷ Dihydroxyacetone 3-phosphate + NADH+ H^+

SPECIFICATION

State	:	Lyophilized
Specific activity	:	more than 7 U/mg protein

PROPERTIES

Molecular weight	:	ca. 73,600
Subunit molecular weight	:	ca. 36,800
Optimum pH	:	9.0 .....(Fig. 1)
pH stability	:	6.5 - 10.0 .....(Fig. 2)
Optimum temperature	:	above 60 °C.....(Fig. 3)
Thermal stability	:	No detectable decrease in activity up to 80 °C. .....(Fig. 4)
Michaelis constants	:	(90 mM Bicine buffer pH 9.0, at 37 °C)
		Glycerol-3-phosphate .....0.119 mM
		NAD^+.....0.036 mM

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for enzymatic determination of glycerol and triglyceride when coupled with glycerokinase.

ASSAY

PRINCIPLE

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

Glycerol-3-phosphate + NAD^+ $\xrightarrow{\text{G3PDH}}$ Dihydroxyacetone phosphate + NADH + H^+

UNIT DEFINITION

One unit of activity is defined as the amount of G3PDH that forms 1 µmol of NADH per minute at 37 °C.

SOLUTIONS

1. Buffer solution ; 100 mM Bicine, pH 9.0
2. Glycerol-3-phosphate solution ; 50 mM (0.172 g Glycerol-3-phosphate bis(cyclohexylammonium) salt/20 mL distilled water)
3. NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid/10 mL distilled water)

PREPARATION OF ENZYME SOLUTION

Dissolve the lyophilized enzyme with distilled water and dilute the enzyme solution to 0.3 – 1.2 U/mL with 50 mM Bicine buffer pH 9.0 containing 0.1 % bovine serum albumin.

PROCEDURE

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

Solution 1:	27.00 mL	Solution 3:	0.50 mL
Solution 2:	1.00 mL	Solution 4:	1.50 mL

2. Incubate at 37 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

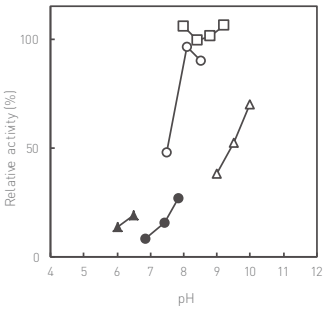


Fig. 1 pH profile

△ CHES, ○ Tris-HCl, ▲ MES
□ Bicine ● phosphate

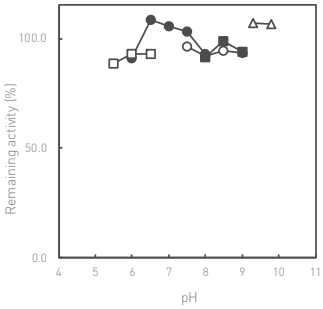


Fig. 2 pH stability

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

△ Gly-Hydrazine, ○ Tris-HCl, ● phosphate, □ MES, ■ Bicine

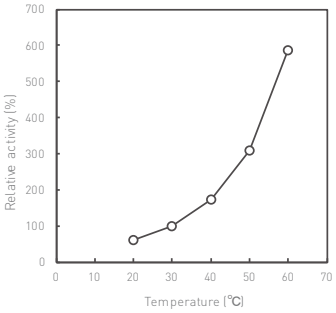


Fig. 3 Thermal stability

defined as 100% at 30 °C.

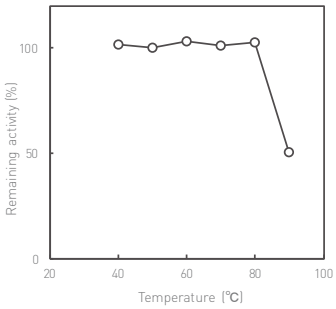


Fig. 4 Thermal stability

treated for 15 min in 0.1 M Bicine Buffer (pH 9.0)

Leucine Dehydrogenase

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

LeuDH

EC 1. 4. 1. 9

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.

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 250 mM Sodium phosphate, pH 10.5
2. 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)
3. 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 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 1:	15.00 mL	Solution 3:	0.93 mL
Solution 2:	10.00 mL	H_2O:	4.07 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

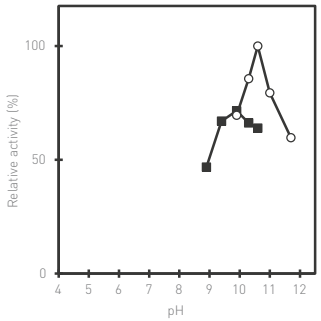


Fig. 1 pH profile
■ Gly-KOH, ○ phosphate

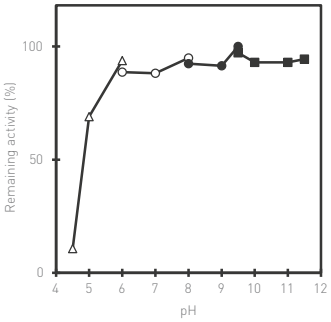


Fig. 2 pH stability
treated for 24 hr at 4 °C in the following buffer solution (0.1 M)
△ acetate, ○ phosphate, ● Tris-HCl, ■ Gly-KOH

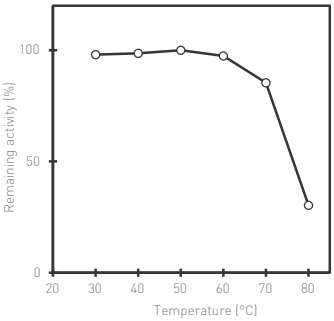


Fig. 3 Thermal stability
treated for 15 min in 0.1 M Gly-KOH buffer, pH 9.0

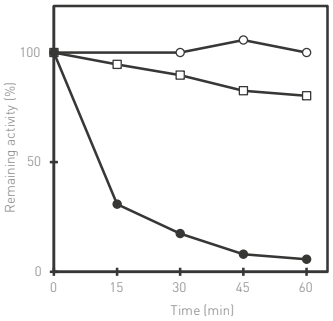


Fig. 4 Thermal stability
treated in 0.1 M Gly-KOH buffer, pH 9.0
○ 60 °C, □ 70 °C, ● 80 °C

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

Malate Dehydrogenase

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

MDH

EC 1. 1. 1. 37

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.

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

UNIT DEFINITION

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

SOLUTIONS

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

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	13.50 mL	Solution 3:	0.57 mL
Solution 2:	1.00 mL	H_2O:	14.93 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

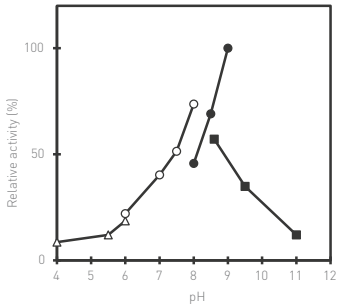


Fig. 1 pH profile

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

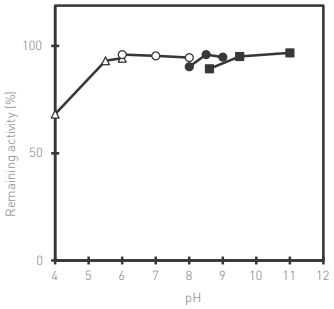


Fig. 2 pH stability

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

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

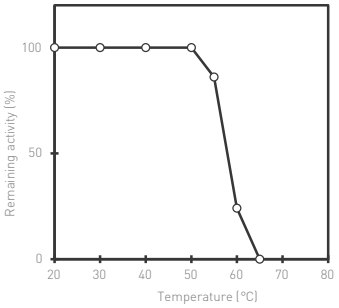


Fig. 3 Thermal stability

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

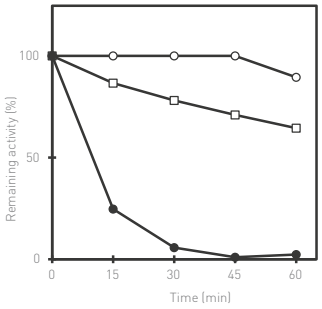


Fig. 4 Thermal stability

treated in 0.1 M Tris-HCl buffer, pH 9.0

○ 50 °C, □ 55 °C, ● 60 °C

Mutarotase

α-D-glucose ⟷ β-D-glucose

MRO

EC 5. 1. 3. 3

Microorganism

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.

sucrose $\xrightarrow{\text{β-Fructosidase}}$ α-glucose + fructose

α-glucose $\xrightarrow{\text{MRO}}$ β-glucose

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

UNIT DEFINITION

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

SOLUTIONS

1. HEPES buffer ; 50 mM (1.19 g HEPES / 100 mL distilled water, adjust pH to 7.5 with NaOH)
2. Sucrose solution ; 16.7 mM (57 mg Sucrose / 10 mL distilled water)
3. NAD^+ solution ; 100 mM (0.663 g NAD^+ free acid / 10 mL distilled water)
4. Glucose dehydrogenase solution ; 3 kU/mL (GlcDH2, Nipro Corp. / 20 mM potassium phosphate containing 2 M NaCl, pH 6.5)
5. β-Fructosidase solution ; ≥ 30 kU/mL (100 mg Invertase from baker's yeast, Sigma-Aldrich I4504 / 1 mL distilled water)

PREPARATION OF ENZYME SOLUTION

Dissolve the lyophilized enzyme with distilled water and dilute to 0.7 to 1.4 U/mL with the enzyme dilutent (20 mM potassium phosphate pH 7.3 containing 1 mg/mL BSA).

PROCEDURE

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

Solution 1:	19.90 mL	Solution 3:	0.60 mL
Solution 2:	1.00 mL	Solution 4:	0.166 mL

2. Add 0.015 mL of the enzyme solution into the cuvette and mix.
3. Incubate at 25 °C for about 3 minutes.

4. Add 0.06 mL of the Solution 5 into the cuvette and mix.
5. Read absorbance change at 340 nm per minute (ΔAbs1) in the linear portion of curve.
6. Run the procedure 1 to 5 with the enzyme diluent instead of the enzyme solution (ΔAbs2).

CALCULATION

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

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

d.f.: dilution factor
6.22: millimolar extinction coefficient of NADH (cm²/μmol)
10: conversion factor
*Protein concentration ; determined by the absorbance at 280 nm (Abs_{280}), where 1 Abs_{280} = 1 mg/mL

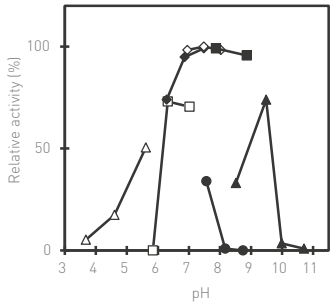


Fig. 1 pH profile

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

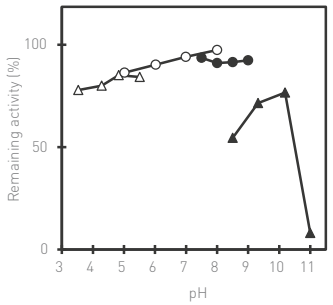


Fig. 2 pH stability

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

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

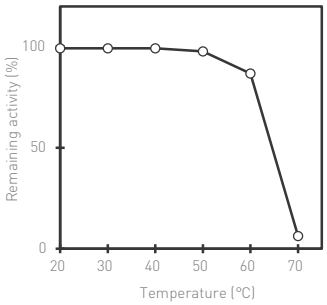


Fig. 3 Thermal stability

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

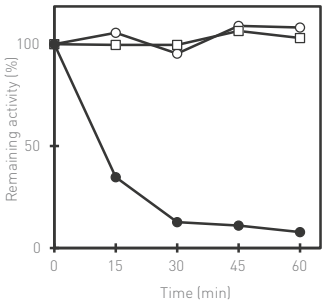


Fig. 4 Thermal stability

treated in 0.1 M potassium phosphate buffer pH 6.5, 0.1 % BSA at

○ 40 °C, □ 50 °C, ● 60 °C

Phenylalanine Dehydrogenase

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

PheDH

EC 1.4.1.20

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 (Do not freeze)

ASSAY

PRINCIPLE

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 400 mM Gly-KCl-KOH, pH 11.0
2. L-Phenylalanine solution ; 100 mM (0.165 g L-phenylalanine/10 mL distilled water)
3. 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 1:	15.00 mL	Solution3:	0.15 mL
Solution 2:	3.00 mL	H_2O:	11.85 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

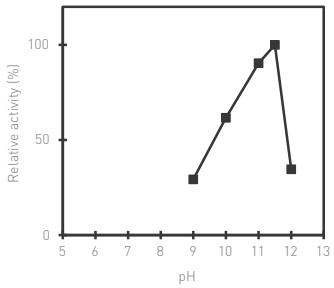


Fig. 1 pH profile
■ Gly-KCl-KOH

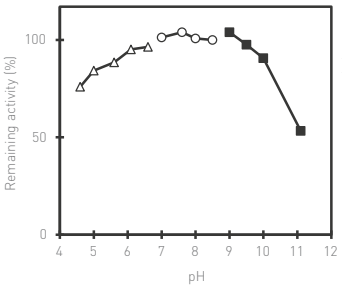


Fig. 2 pH stability
treated for 24 hr at 4 °C in the following buffer solution (50 mM)
△ acetate, ○ phosphate
■ Gly-KCl-KOH

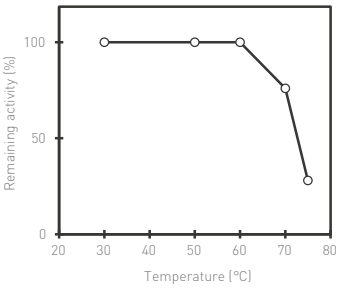


Fig. 3 Thermal stability
treated for 15 min in 10 mM potassium phosphate buffer, pH 7.2

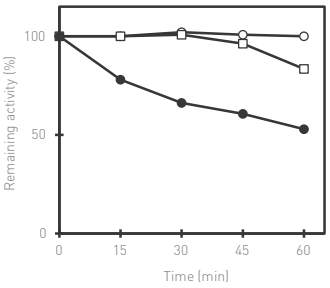


Fig. 4 Thermal stability
treated in 10 mM potassium phosphate buffer, pH 7.2
○ 50 °C, □ 60 °C, ● 70 °C

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

Phosphoglucose Isomerase

D-Glucose-6-phosphate ⟷ D-Fructose 6-phosphate

PGI

EC 5. 3. 1. 9

Bacillus stearothermophilus

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 : (95 mM Tris-HCl buffer, pH 9.0, at 30 °C)

Fructose-6-phospate 0.27 mM

STORAGE

Stable at -20 °C for at least one year

ASSAY

PRINCIPLE

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

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

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

UNIT DEFINITION

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

SOLUTIONS

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

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	28.44 mL	Solution 3:	0.60 mL
Solution 2:	0.90 mL	Solution 4:	0.06 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of the curve.

CALCULATION

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

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

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

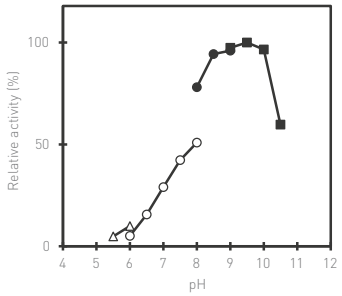


Fig. 1 pH profile

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

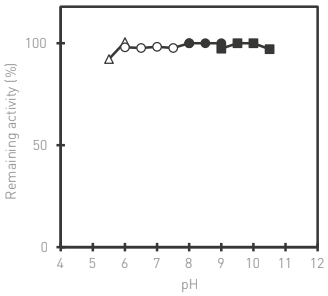


Fig. 2 pH stability

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

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

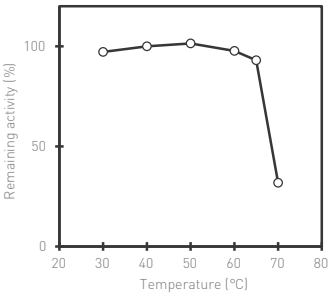


Fig. 3 Thermal stability

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

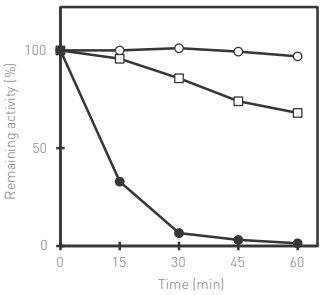


Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.5

○ 60 °C, □ 65 °C, ● 70 °C

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

Phosphotransacetylase

Acetyl-CoA + Pi ⟷ Acetylphosphate + CoA

PTA

EC 2. 3. 1. 8

Bacillus stearothermophilus

SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at -20 °C for at least one year

APPLICATION

The enzyme is useful for determination of CoA or acetate.

ASSAY

PRINCIPLE

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

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

UNIT DEFINITION

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

SOLUTIONS

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

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	26.0 mL	Solution 3:	1.0 mL
Solution 2:	2.0 mL	Solution 4:	1.0 mL

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

CALCULATION

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

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

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

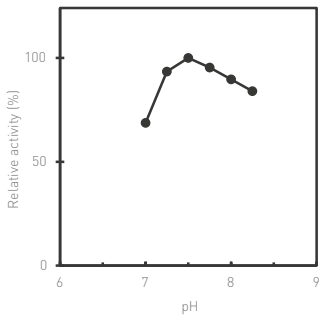


Fig. 1 pH profile

● Tris-HCl

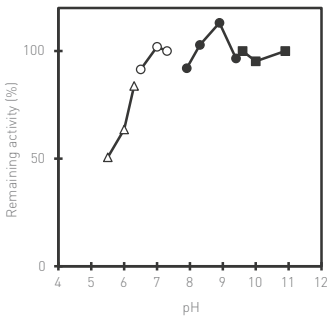


Fig. 2 pH stability

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

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

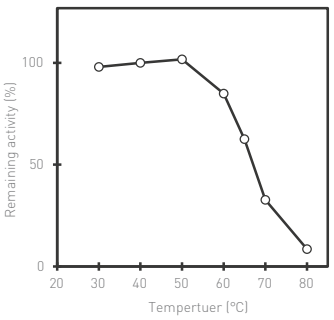


Fig. 3 Thermal stability

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

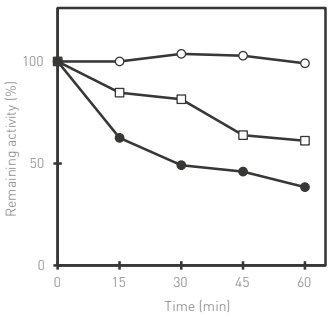


Fig. 4 Thermal stability

treated in 50 mM Tris-HCl buffer, pH 8.0

○ 50 °C, □ 60 °C, ● 65 °C

Polynucleotide Phosphorylase 3

PNPASE3

EC 2. 7. 7. 8

recombinant E.coli

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

For Depolymerization Reaction

SPECIFICATION

State	:	Frozen liquid (50mM Tris HCl buffer pH9.0)
Specific activity	:	more than 2,000 U/mg protein

PROPERTIES

Molecular weight	:	300,000 - 340,000
Subunit molecular weight	:	ca. 85,000
Optimum pH	:	9.0 .....(Fig. 1)
pH stability	:	8.5 - 10.0 .....(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 8.5, at 60 °C)
		Poly A .....0.27 mM**
		KH_2PO_4 .....3.0 mM
		**concentration of poly A was calculated as AMP concentration
Effectors	:	cations and anions.....(Fig. 5, 6)

STORAGE

Stable at -20 °C

APPLICATION

The enzyme is useful for the preparation of polyribonucleotide.

ASSAY

PRINCIPLE

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

Poly A_n + Pi $\xrightarrow{PNPase3}$ Poly A_{n-1} + ADP (1)

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

Pyruvate + NADH + H^+ $\xrightarrow{LD}$ Lactate + NAD^+ (2)

UNIT DEFINITION

One unit of activity is defined as the amount of PNPase3 that forms 1 µmol of ADP per hour at 60 °C by depolymerizing of Poly A.

SOLUTIONS

Reaction 1 :

1. Buffer solution ; 100 mM Tris-HCl, pH 9.5 ((1.212 g Tris + 0.074 g EDTA + 0.014 mL 2-mercaptoethanol + 0.610 g $MgCl_2 \cdot 6H_2O$ + 0.746 g KCl)/80 mL distilled water, adjusted to pH 9.5 with 1 N HCl and filled up to 100 mL with distilled water)
2. KH_2PO_4 solution ; 65 mM (0.088 g KH_2PO_4/10 mL distilled water)
3. polyadenylate (Poly A) solution ; (25 mg Poly A potassium salt/1 mL distilled water; ca. 35 mM based on AMP concentration)

Reaction 2 :

4. Buffer solution ; 100 mM Triethanolamine buffer, pH 7.6 ((9.300 g triethanolamine-HCl + 0.407 g $MgCl_2 \cdot 6H_2O$ + 0.373 g KCl)/400 mL distilled water, adjusted to pH 7.6 with 1 N NaOH and filled up to 500 mL with distilled water)
5. NADH solution ; 13.1 mM (0.100 g NADH disodium salt·$3H_2O$/10 mL distilled water)
6. Phosphoenolpyruvate (PEP) solution ; 56 mM (0.150 g PEP MCA salt/10 mL distilled water)
7. Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M $(NH_4)_2SO_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C
8. Lactate dehydrogenase (LDH) ; (from pig heart, Oriental Yeast Co. Ltd., Product Code: LDH-02) ammonium sulfate suspension, approx. 1,000 U/mL at 25 °C

PREPARATION OF ENZYME SOLUTION

Dilute the concentrated enzyme solution to 1 to 5 U/mL with 50 mM Tris HCl buffer, pH 8.5.

PROCEDURE

Reaction 1 :

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

Solution 1:	2.50 mL	Solution 3:	1.00 mL
Solution 2:	1.00 mL	H_2O:	1.00 mL

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 2 :

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

Solution 4:	24.18 mL	Solution 7:	0.12 mL
Solution 5:	0.40 mL	Solution 8:	0.05 mL
Solution 6:	0.25 mL		

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

Repeat the Procedure using blank (Abs•blank).

CALCULATION

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

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

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

REFERENCE : 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)

For Polymerization Reaction

ASSAY

PRINCIPLE

The amount of inorganic phosphate is measured according to the following reaction.

nADP $\xrightarrow{\text{PNPase3}}$ Poly A + nPi

UNIT DEFINITION

One unit of activity is defined as the amount of PNPase3 that forms 1µmol of inorganic phosphate per 15 minutes at pH 9.0, 60 °C.

SOLUTIONS

Reaction mixture :

Desolve 747.45 mg Tris and 12.38 mg BSA by distilled water 20mL. Add 5mL of 5mM EDTA solution. Adjust pH to 10.0 by 1 N HCl. Then add 534mg ADP and 1.56mL of 0.2M $MgCl_2$. Adjust pH to 9.0 and fill up to 50mL by distilled water.

PREPARATION OF ENZYME SOLUTION

Dilute the concentrated enzyme solution to 1 to 5 U/mL with 50 mM Tris HCl buffer, pH 8.5.

PROCEDURE

Mix 20 µL of diluted enzyme solution and 80 µL of preincubated reaction mixture and incubate at 60 °C. After exactly 15 min, stop the reaction by adding 900 µL of 4 % PCA (Perchloric acid).

Measure the concentration of the inorganic phosphate by chemical method molybdic acid color reaction) At the same time, repeat procedure using diluent as blank.

CALCULATION

$$\text{Volume activity (U/mL)} = (A_{sample} - A_{blank}) \times \frac{1000}{20} \times \text{d.f.}$$

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

A; concentration of inorganic phosphate (mM)
d.f.: dilution factor
*Protein concentration; determined by the absorbance at 280nm (Abs280) where 1 Abs280 = 1 mg/mL

One unit of polymerization reaction is equivalent to approximately 16.9 unit of depolymerization reaction.

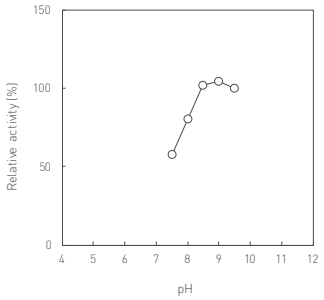


Fig. 1 pH profile

○ Tris-HCl

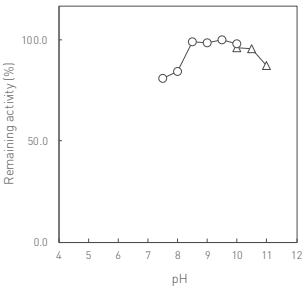


Fig. 2 pH stability

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

△ Gly-KOH, ○ Tris-HCl

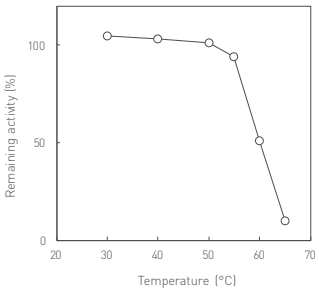


Fig. 3 Thermal stability

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

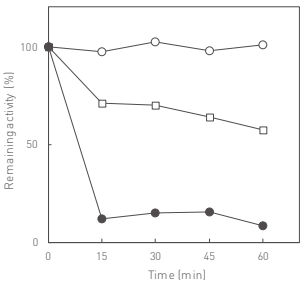


Fig. 4 Thermal stability

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

○ 55 °C, □ 60 °C, ● 65 °C

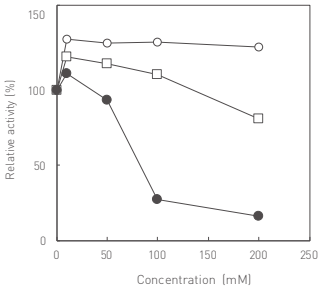


Fig. 5 Effect of various cations on the activityof PNPase3 in the following Assay Method

Measurement : 0.015 mL of each cation solution, 0.010 mL of enzyme solution and 0.055 mL of reaction mixture were mixed, and reacted at 60 °C.After 10 minutes, the quantity of ADP was determined

○ NaCl, □ KCL, ● $MgCl_2$

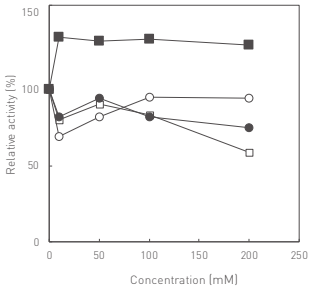


Fig. 6 Effect of various anions on the activity ofPNPase3 in the following Assay Method

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

○ CH_3COONa, □ Na_2SO_4, ● NaH_2PO_4, ■ NaCl

Pyruvate Kinase

ATP + Pyruvate ⟷ ADP + Phosphoenolpyruvate

PK

EC 2.7.1.40

Bacillus stearothermophilus

SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at -20 °C for at least one year

APPLICATION

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

ASSAY

PRINCIPLE

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

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

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

UNIT DEFINITION

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

SOLUTIONS

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

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1: 22.71 mL	Solution 3: 0.45 mL	Solution 5: 0.48 mL	Solution 7: 0.06 mL
Solution 2: 2.40 mL	Solution 4: 3.00 mL	Solution 6: 0.90 mL	

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 340 nm per minute (ΔAbs_{340}) in the linear portion of curve.

CALCULATION

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

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

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

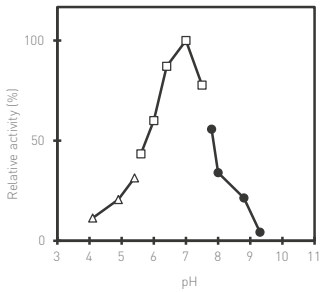


Fig. 1 pH profile

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

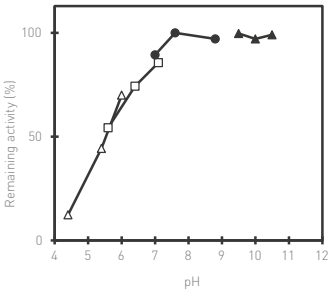


Fig. 2 pH stability

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

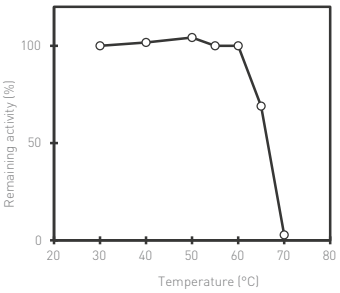


Fig. 3 Thermal stability

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

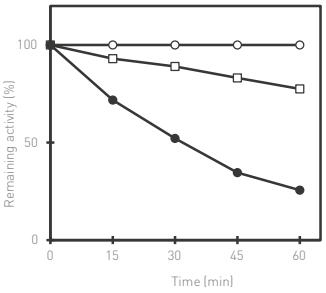


Fig. 4 Thermal stability

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

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

Superoxide Dismutase

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

SOD

EC 1.15.1.1

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.

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

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

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

UNIT DEFINITION

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

SOLUTIONS

1. Buffer solution ; 75 mM Potassium phosphate buffer, pH 7.8
2. Xanthine solution ; 0.75 mM (0.010 g xanthine/50 mL N/250 NaOH)
3. Cytochrome c solution ; 0.15 mM (0.019 g cytochrome C/10 mL distilled water, Sigma-Aldrich Co., No. C-2506, from horse heart)
4. EDTA solution ; 1.5 mM (0.028 g EDTA disodium salt·$2H_2O$/50 mL distilled water)
5. Xanthine oxidase (XOD) ; (from buttermilk, Sigma-Aldrich Co., No. X-1875) suspension in 2.3 M $(NH_4)2SO_4$ solution is diluted to 0.04 U/mL with distilled water. (prepare freshly)

PREPARATION OF ENZYME SOLUTION

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

PROCEDURE

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

Solution 1:	22.00 mL	Solution 3:	2.00 mL
Solution 2:	2.00 mL	Solution 4:	2.00 mL

2. Incubate at 30 °C for about 3 minutes.
3. Add 0.20 mL of Solution 5 into the cuvette and mix.
4. Read absorbance change at 550 nm per minute for the linear portion of curve (ΔAbs•test)*.
5. Add 0.005 mL of Solution 1 in place of enzyme solution and measure the same above 4 (ΔAbs•blank).

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

CALCULATION

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

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

d.f.: dilution factor
*Protein concentration ; determined by the absorbance at 280nm (Abs280), where 1 Abs280 = 1 mg/mL

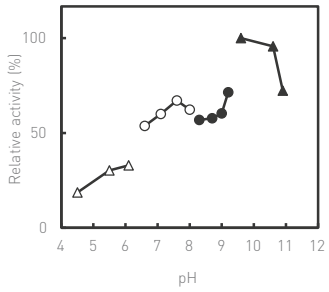


Fig. 1 pH profile

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

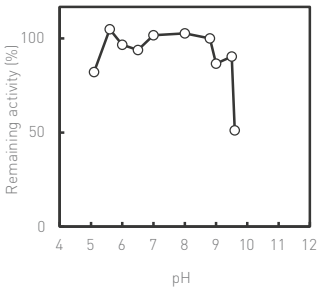


Fig. 2 pH stability
treated for 24 hr at 4 °C in the Britton-Robinson buffer

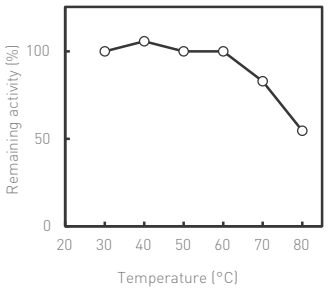


Fig. 3 Thermal stability
treated for 15 min in 0.1 M potassium phosphate buffer, pH 7.5

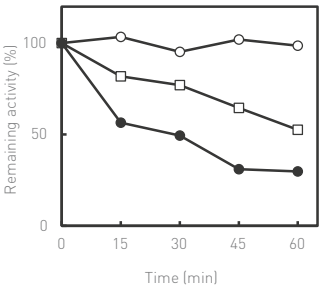


Fig. 4 Thermal stability
treated in 0.1 M Tris-HCl buffer, pH 8.5

○ 60 °C, □ 70 °C, ● 80 °C

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