NIPRO ENZYMES

DIAPHORASE 3 (DI-3)

[EC 1. 6. 99. -]

from recombinant *E. coli*

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

SPECIFICATION

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

PROPERTIES

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

STORAGE

Stable at -20 to 5 °C for one year

APPLICATION

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

ASSAY

Principle

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

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

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 500 mM Tris-HCl, pH 8.5
Ⅱ NADH solution ; 13.1 mM (0.100 g NADH disodium salt·3H_2O/10 mL distilled water)
Ⅲ 2,6-Dichlorophenolindophenol (DCIP) solution ; 1.2 mM (2.0 mg DCIP sodium salt·2H_2O/5mL distilled water) (prepare freshly)

Preparation of Enzyme Solution

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

Procedure

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

Solution Ⅰ	3.00 mL
Solution Ⅱ	2.28 mL
H_2O	23.22 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

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

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

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

REFERENCE

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

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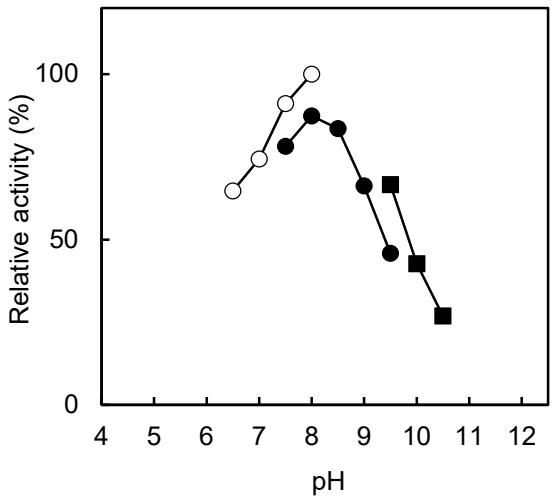

Fig. 1 pH profile

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

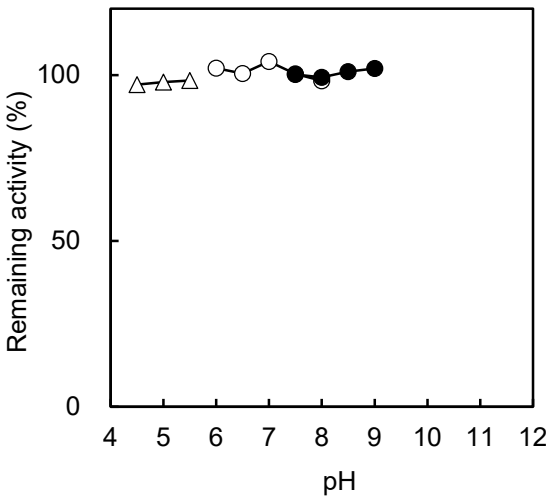

Fig. 2 pH stability

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

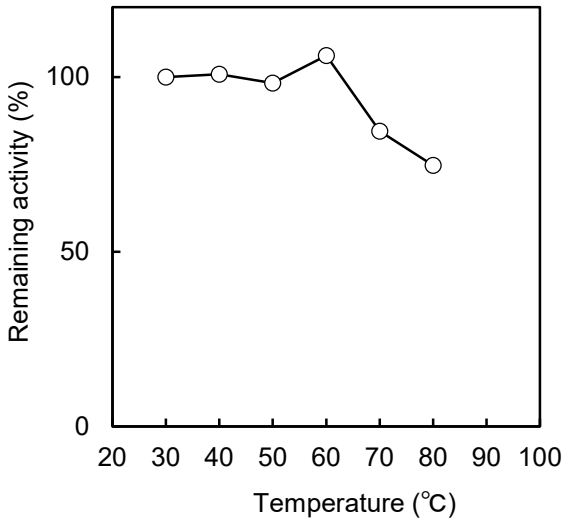

Fig. 3 Thermal stability

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

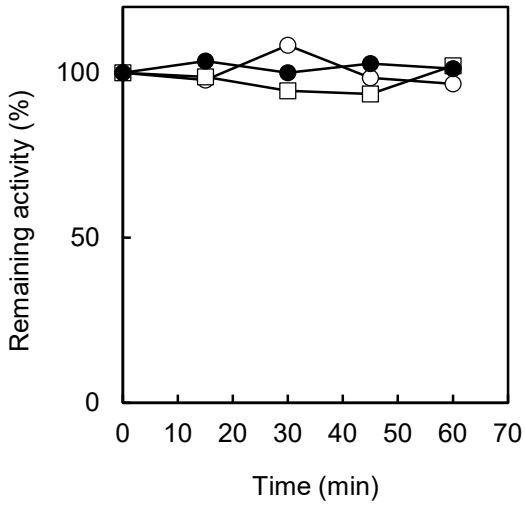

Fig. 4 Thermal stability

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

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Table 1. SUBSTRATE SPECIFICITY OF DIAPHORASE

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

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

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

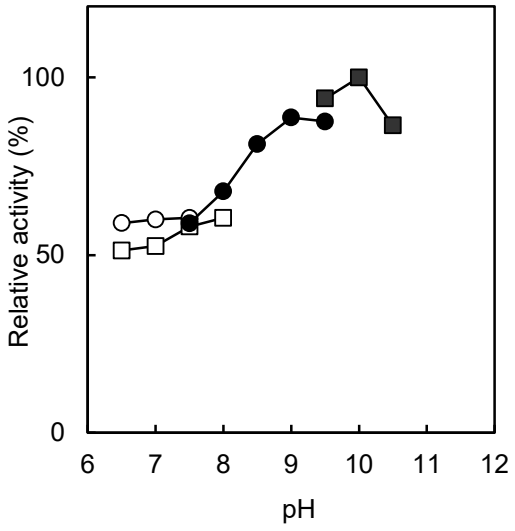

Fig. 5 pH profile (NTB)

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

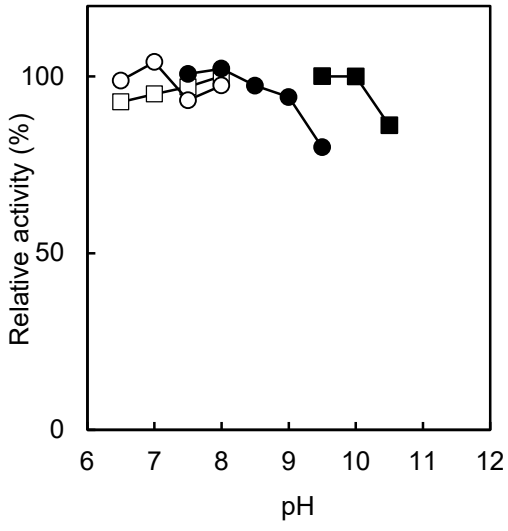

Fig. 6 pH profile (MTT)

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