NIPRO ENZYMES

DIAPHORASE 22 (Di-22)

[EC 1. 8. 1. 4]

from recombinant *E.coli*

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

SPECIFICATION

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

PROPERTIES

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

STORAGE

Store at -20℃

APPLICATION

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

ASSAY

Principle

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

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

Unit Definition

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

Solutions

Ⅰ Buffer solution ; 100 mM HEPES, pH 7.0

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

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

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

Preparation of Enzyme Solution

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

Procedure

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

Solution Ⅰ	15.00 mL	SolutionⅣ	1.50 mL
Solution Ⅱ	1.50 mL	H_2O	10.80 mL
SolutionⅢ	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

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

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

d.f. ; dilution factor

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

*Protein concentration ; determined by Bradford's method

REFERENCE

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

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Table 1. SUBSTRATE SPECIFICITY OF DIAPHORASE 22

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

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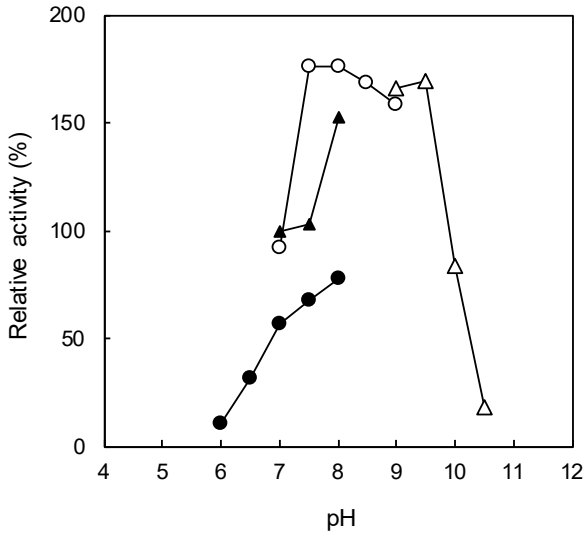

Fig. 1 pH profile

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

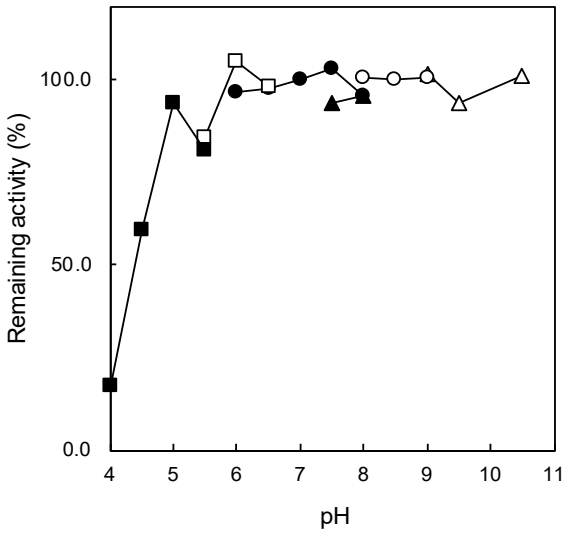

Fig. 2 pH stability

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

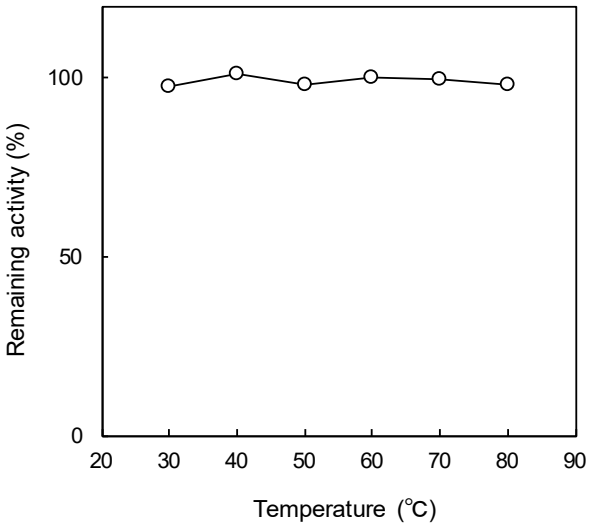

Fig. 3 Thermal stability

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

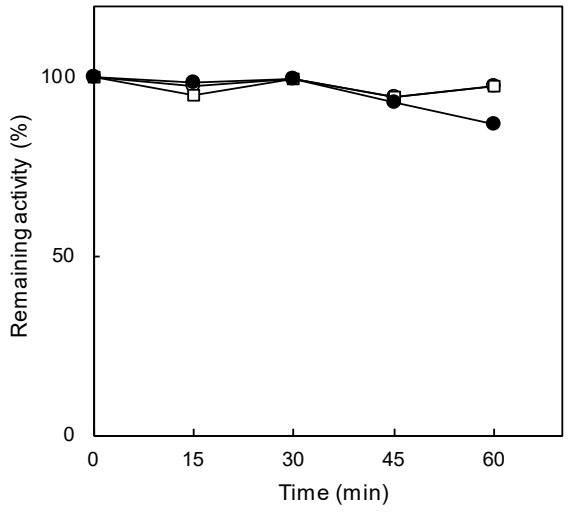

Fig. 4 Thermal stability

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