LEADGENE
LEADGENE BIOMEDICAL, INC.

Product Information & Manual

Information of other products is available at: www.leadgenebio.com

Diaphorase

Cat no. LDG0021RG

Product Overview

Description

Diaphorase, also known as NADH dehydrogenase or NAD(P)H oxidoreductase, is an essential enzyme involved in cellular redox reactions. It facilitates electron transfer from NADH or NADPH to various acceptors, playing a crucial role in the electron transport chain. This enzyme helps mitigate oxidative stress by reducing harmful oxidants and is key in regulating metabolic pathways by maintaining NAD^+/NADH and $NADP^+$/NADPH ratios. Additionally, diaphorase contributes to cellular signaling processes, influencing cell proliferation, differentiation, and apoptosis.

Expression system

Escherichia coli

Specification

Appearance	Yellow amorphous powder, lyophilized
Activity	500 U/mg or more (containing approx. 10% of stabilizers)

Properties

Stability	Stable at −20℃ for at least one year
Isoelectric point	7.82

Reconstitution

It is recommended to weigh and reconstitute 10 mg of lyophilized powder in 250 µL double-distilled water directly and incubate the solution for at least 10 mins to ensure sufficient re-dissolved.

Applications

1. Biotransformation [1]
2. Biosensor design [2]
3. Colorimetric determination of NAD(P)H and many dehydrogenases in combination with various dyes that act as hydrogen acceptors from NAD(P)H

Assay

1. Assay principle

$$NADH + H^+ + DCPIP \xrightarrow{\text{Diaphorase}} NAD^+ + \text{Leucodye}$$

Reduction of 2,6-dichlorophenol-indophenol (DCPIP) is measured at 600 nm by spectrophotometry.

2. Unit definition

One unit causes decrease in DCPIP by one unit of absorbance (1.0) per minute under the following conditions : 27 mM Tris-HCl pH 7.5, 0.2 mM NADH, 40 µM DCPIP and 33 µg/ mL BSA.

3. Reagents

A. Buffer solution	0.2 M Tris-HCl, pH 7.5 (MW: 157.6, 1.576 g in 50 mL MQ)
B. NADH solution	6.0 mM (Prepare freshly and store on ice) (MW: 709.4, 0.021 g in 5 mL MQ)

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

C. DCPIP solution	1.2 mM (MW: 290.08) [12 mM (0.0348 g in 10 mL MQ) was prepared first, and it was diluted to 1.2 mM for use.]
D. Enzyme diluent	Buffer solution (A) containing 0.1% of bovine serum albumin [0.05 g of BSA was dissolved in 50 mL of Buffer solution (A)]

4. Procedure

(1) Prepare the following Working Solution in a cuvette (d = 1.0 cm) and equilibrate at 25℃ for about 5 minutes (for 4 reactions).

Working Solution

H_2O	4.8 mL
Buffer solution (Reagent A)	0.6 mL
NADH solution (Reagent B)	0.2 mL
Total	**5.6 mL**

(2) Pipette 1.4 mL of working solution into a tube.

(3) Add **0.05 mL** each of the enzyme solution* and DCPIP solution (**Reagent C**) in this order and mix by rapid inversion.

(4) Pipette 1 mL of the mixture into a cuvette (d=1.0 cm).

Concentration in a reaction	
Tris-HCl	27 mM
NADH	0.2 mM
BSA	33 μg/ mL
DCPIP	40 μM

(5) Record the decrease in optical density at 600 nm against water for 1 to 5 minutes with a spectrophotometer at room temperature and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test). At the same time, measure the blank rate (ΔOD blank) using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.

* Dilute the enzyme to **0.4–0.8 U/mL** with ice-cold enzyme diluent (**Reagent D**) and store on ice.

(6) Activity can be calculated by using the following formula:

Volume activity (U/mL) =

$$\frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times df}{\frac{1.0 \times Vs}{}}$$

$$= \Delta OD/min \times 20 \times df$$

Weight activity (U/mg) = (U/mL) × 1/C

Vs: Sample volume (0.05 mL)

1.0: Unit absorbance at 600 nm due to unit definition

df: Dilution factor

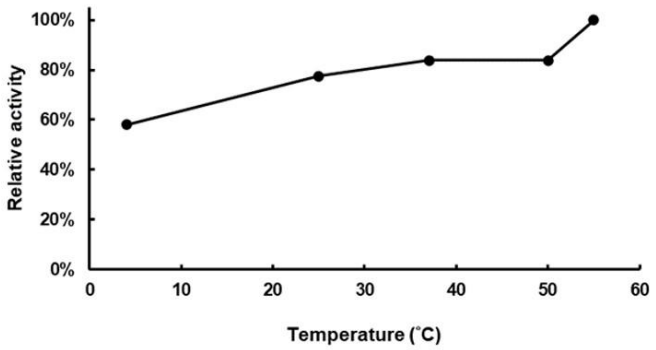
C: Enzyme concentration in dissolution (mg/mL)

References

1. *Bharat Bhushan. et al.* Diaphorase catalyzed biotransformation of RDX via N-denitration mechanism. *Biochemical and Biophysical Research Communications* (2002).
2. *R. Antiochia" . et al.* Purification and sensor applications of an oxygen insensitive, thermophilic diaphorase. *Analytica Chimica Acta* (1997).

The effect of different conditions on Diaphorase

A.



LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

Figure A. Temperature activity of Diaphorase. The enzyme reactions in 0.2 M Tris-HCl buffer, pH 7.5, were carried out under different temperatures.

B.

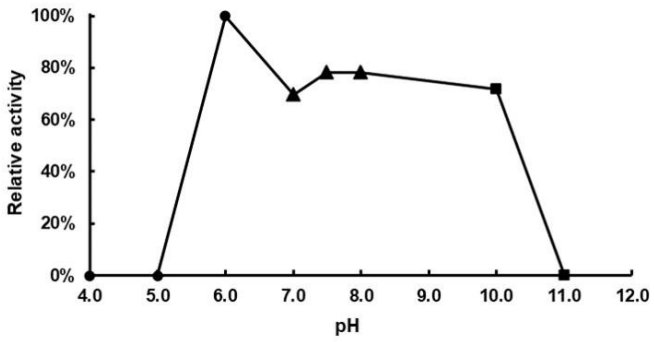


Figure B. pH activity of Diaphorase. The buffer conditions with various pH values were used in the reaction at 25°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

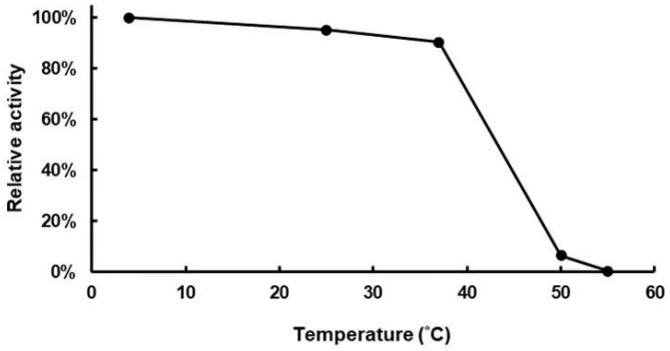


Figure C. Thermal stability of Diaphorase. The enzyme powder was reconstituted by double-distilled water and treated with different temperatures for 30 minutes. Final concentration: 48 U/mL

D.

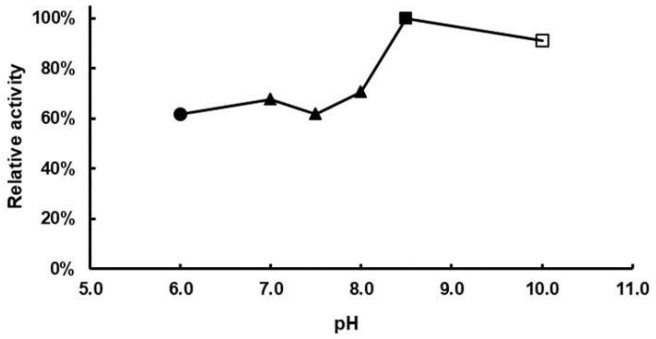


Figure D. pH stability of Diaphorase. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 3 hours at 30°C. pH 6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0, 0.1 M Carbonate-bicarbonate buffer.

E.

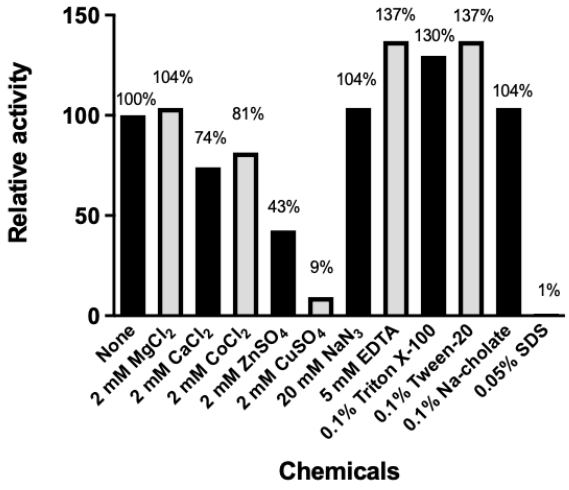


Figure E. The effects of various chemicals on Diaphorase. The enzyme was incubated with the chemicals at 25°C for 1 hour.

<u>Disclaimer</u>

For Research Use or Further Manufacturing Only.

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan