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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Diaphorase

Cat no. LDG0021RG

Product Overview

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
Molecular weight	25.9 kDa
Isoelectric point	7.82

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 [3]

Assay

1. Assay principle

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

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 conditions detailed below.

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)
C. DCPIP solution	1.2 mM (MW: 290.08) [2.4 mM (0.007 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** 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.

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

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

(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) =

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

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

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).
3. TOYOBO Biotechnology Operating Department

The effect of different conditions on Diaphorase

A.

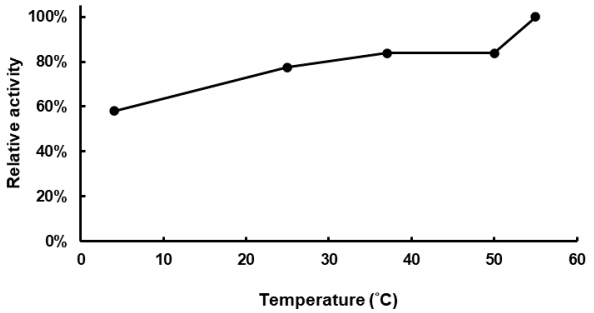

Figure A. Temperature activity of Diaphorase. The enzyme reactions in 0.2 M Tris-HCl buffer, pH 7.5, were carried out under different temperature.

B.

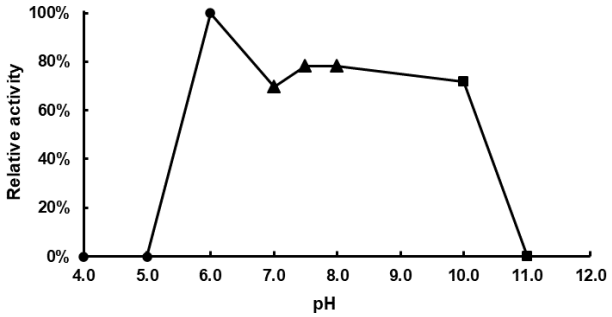

Figure B. pH activity of Diaphorase. The buffer conditions with various pH values were used in the reaction at 25°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

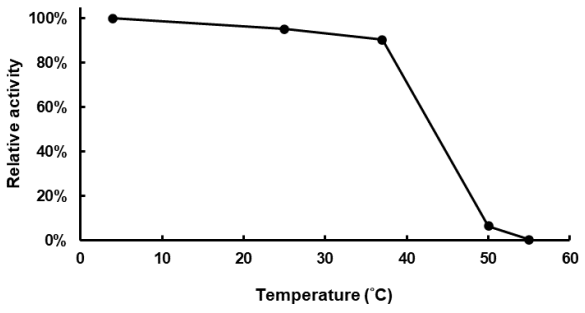

Figure C. Thermal stability of Diaphorase. The enzyme powder was reconstituted by double-distilled water and treated with

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different temperature for 30 minutes. Final concentration: 48 U/ mL

D.

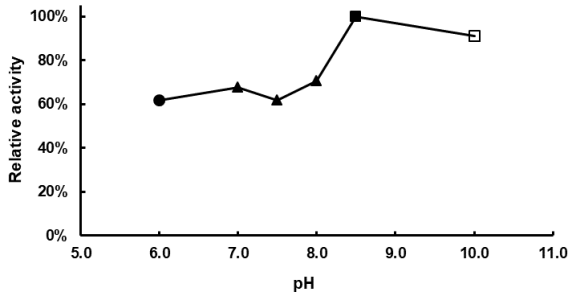

Figure D. pH stability of Diaphorase. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 3 hours at 30°C. pH 6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0, 0.1 M Carbonate-bicarbonate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

Disclaimer

This product is for research use only and is not intended for diagnostic use.