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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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NADH Oxidase

Cat no. LDG0038RG

Product Overview

Description

NADH Oxidase is a flavoprotein enzyme that catalyzes the oxidation of NADH to NAD^+, coupled with the reduction of molecular oxygen (O_2) to either hydrogen peroxide (H_2O_2) or water (H_2O), depending on the enzyme source and reaction conditions. This enzyme plays a key role in redox homeostasis and regenerating NAD^+ for metabolic processes such as glycolysis and fermentation.

Expression system

Escherichia coli

Specification

Appearance	Yellow powder, lyophilized
Activity	200 U/mg or more

Properties

Stability	Stable at −20℃ for at least one year
Isoelectric point	8.92

Reconstitution

It is recommended to weigh and reconstitute 10 mg of lyophilized powder in 200 µL double-distilled water directly and incubate the solution for at least 10 mins to ensure sufficient re-dissolved.

Applications

1. Biosensor design [1]
2. Cofactor regeneration [2]

Assay

1. Assay principle

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

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)
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

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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.

Concentration in a reaction	
Tris-HCl	27 mM
NADH	0.2 mM
BSA	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 **0.3–0.8 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. *Zhiming Liu. et al.* NADH and glutamate on-line sensors using Os-gel-HRP/GC electrodes modified with NADH oxidase and glutamate dehydrogenase. *Biosensors and Bioelectronics* (1999).
2. *Li-Jian Zho. et al.* Regeneration of cofactor $NAD(P)^+$ with NAD(P)H oxidase for the production of value-added chemicals. *Front. Bioengineering and Biotechnology* (2025).

The effect of different conditions on NADH Oxidase

A.

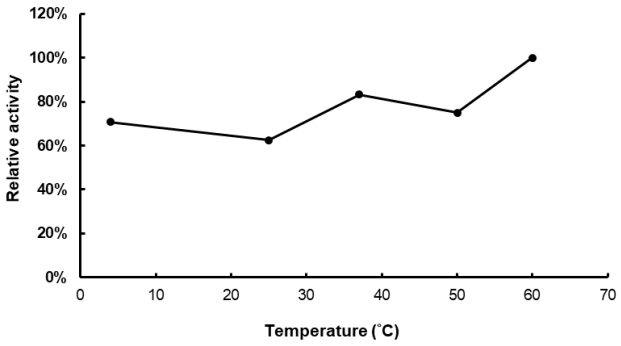

Figure A. Temperature activity of NADH Oxidase. The enzyme reactions in 0.2 M Tris-HCl buffer, pH 7.5, were carried out under different temperatures.

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B.

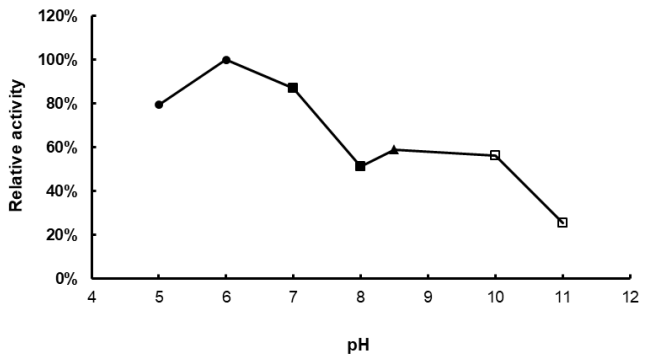

Figure B. pH activity of NADH Oxidase. The buffer conditions with various pH values were used in the reaction at 25°C. pH 5.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

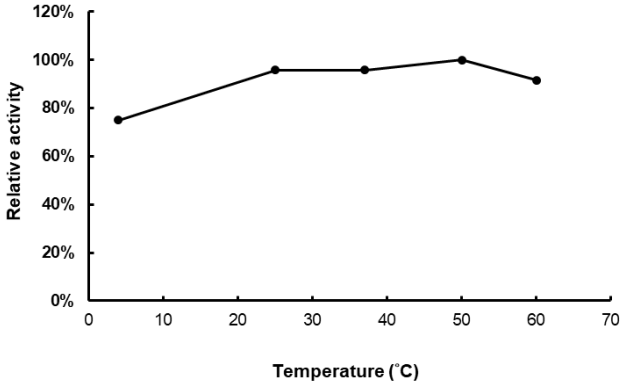

Figure C. Thermal stability of NADH Oxidase. The enzyme powder was reconstituted by double-distilled water and treated with different temperatures for 30 minutes. Final concentration: 20 U/mL

D.

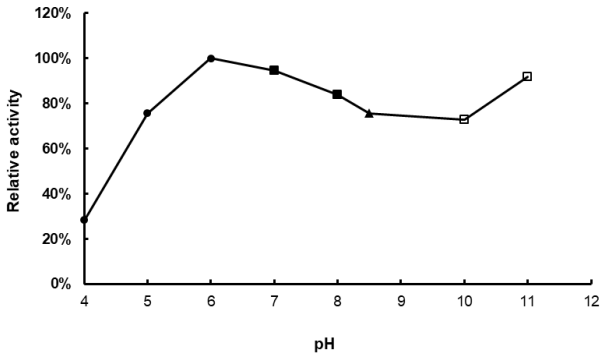

Figure D. pH stability of NADH Oxidase. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 3 hours at 30°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

E.

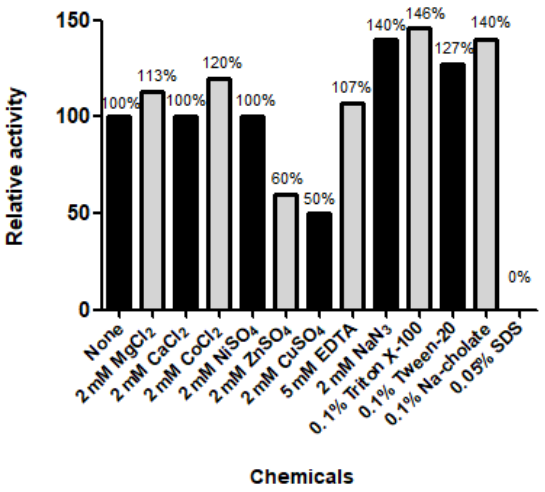

Figure E. The effects of various chemicals on NADH Oxidase. The enzyme was incubated with the chemicals at 25°C for 1 hour.

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