Diaphorase, *Clostridium kluyveri*

CATALOG NO: P1501-1 (1 KU)

ALTERNAME NAMES: *Diaphorase, Lipoamide Dehydrogenase, Lipoyl Dehydrogenase, NAD(P)H dehydrogenase (quinone); EC 1.6.99.2*

SOURCE: *Clostridium kluyveri*

FORM: Lyophilized

PREPARATION: Lyophilized powder (contains no ammonium sulfate). IU per 1 mg powder is approximately 300 units.

SPECIFIC ACTIVITY: ＞20 U/mg (at 25°C and pH 7.5)

STORAGE CONDITION: Store at -20°C

DESCRIPTION: Diaphorase is a flavoprotein widely distributed in animal tissues. It catalyzes the transport of hydrogen from dihydrocodehydrogenase I to acceptors like methylene blue or cytochrome, but not to molecular oxygen. Dehydrogenases are an important target for the development of cancer therapeutics.

ASSAY PROCEDURE: **NAD(P)H + acceptor ＝ NAD(P)+ ＋ reduced acceptor**

a. Spectrophotometric Method:

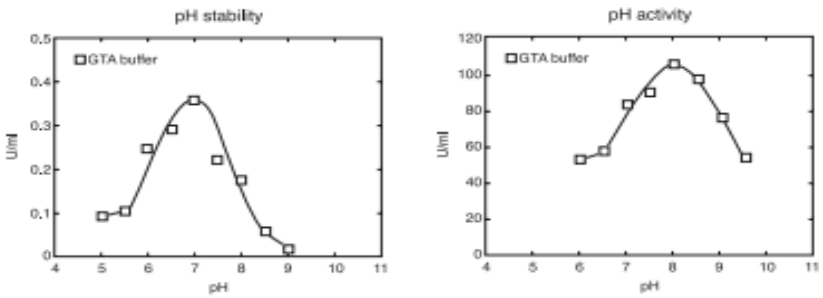
Wavelength；600 nm, Light path length；1 cm, Temperature；25℃
Pipette the following reagents into a cuvette
2.75 mL Potassium phosphate buffer (0.1 mol/L, pH 7.5)
0.10 mL NADH（9 mmol/L) dissoluted in Tris (10 mmol/L)
0.10 mL 2, 6-dichlorophenolindophenol (2.7 mmol/L)
0.05 mL Diaphorase (about 2.5 U/mL)

b. Calculation:

ΔA/min x V x D/ d x v ＝O.D.unit */mL

ΔA/min = The change in absorbance at 600 nm/minute (revise the blank activation of Diaphorase (-))
V = Total volume of reaction mixture (3.00 mL)
D = Enzyme dilution factor
d = Light path length (1 cm)
v = Volume of enzyme sample (0.05 mL)
*O.D.unit：When the absorbance changes 1.0 during activation in 600 nm; we define as 1 O.D unit.

REFERENCE DATA:



RELATED PRODUCTS:

- NADH, disodium salt (2735)
- L-Lysine 6-Oxidase, *M. mediterranea* Recombinant (P1500)
- NADH Oxidase Activity Assay Kit (Colorimetric) (K2028)
- NADH Oxidase Activity Assay Kit (Fluorometric) (K2019)

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