Diaphorase

NAD(P)H dehydrogenase(quinone)

NAD(P)H : (quinone-acceptor) oxidoreductase (EC 1.6.99.2)

from Clostridium kluyveri

Reaction Equation

NAD(P)H + acceptor = $NAD(P)^+$ + reduced acceptor

Specification

Specific Activity

IU/mg protein

Specifications
>20 units※

※O.D. unit, 25℃, pH 7.5

Assay Procedure

Ⅰ. 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)

Ⅱ. Calculation

$$\frac{\Delta A/\min \cdot V \cdot D}{d \cdot v} = \text{O.D.unit}^{※}/\text{mL}$$

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)

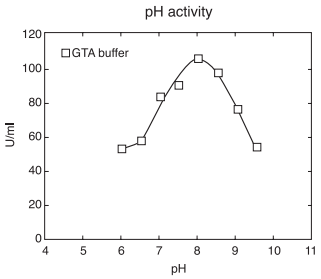
D = Enzyme dilution factor

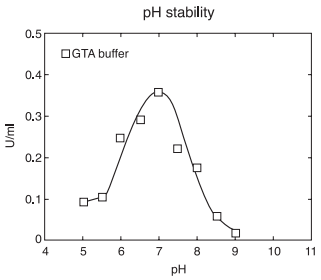
d = Light path length (1 cm)

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





Preparation and storage

Product Code : Diaphorase-03

Lyophilized powder (contains no ammonium sulfate)
……below −20℃

IU per 1 mg powder is approximately 300 units.

OYC No./Package

OYC No.	Package
46445003	1,000 units
46446003	10,000 units
46445903	Bulk

(Research reagent use only, not for medical use.)