rNADS

recombinant NAD synthetase from Bacteria
Deamido-NAD^+: ammonia ligase (AMP-forming) (EC 6.3.1.5)

Reaction Equation

Deamide-NAD^+ + ATP + NH_3 $\rightleftarrows$ NAD^+ + AMP + PPi

Specification

Specific activity
IU/mg protein > 1.3 units

Preparation and Storage

Lyophilized powder
Store below -20˚C

Properties

pH stability : pH 5.5-8.5 (37˚C, 2 weeks)
Thermal stability : ≦60˚C (pH 7.5, 10 min)
Optimum pH : 9.0
Optimum temp. : 45-50˚C
Km value : 3.7×10^{-4} mol/L (NAAD), 4.2×10^{-3} mol/L (NH_4Cl)
Molecular weight : 27 kDa (SDS-PAGE)

Assay Procedure

I. Spectrophotometric Method
Wavelength: 340 nm, Light path length: 1 cm
Final volume: 3.03 mL, Temperature: 37˚C

Pipette the following reagents into a cuvette

1.50 mL	DEA-HCl buffer (0.8 mol/L, pH 9.5)
0.30 mL	$MgCl_2$ (0.1 mol/L)
0.30 mL	NH_4Cl (0.1 mol/L)
0.60 mL	ATP (50 mmol/L)
0.15 mL	NAAD (40 mmol/L)
0.15 mL	G6P (40 mmol/L)
0.01 mL	G6PDH (1,000 U/mL)
0.02 mL	rNADS (approx. 1.5 U/mL)

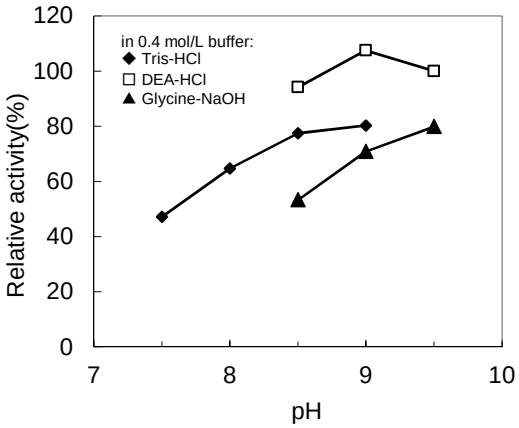
II. Calculation

$$IU/mL = \frac{\Delta A/min \cdot V \cdot D}{6.3 \cdot d \cdot v}$$

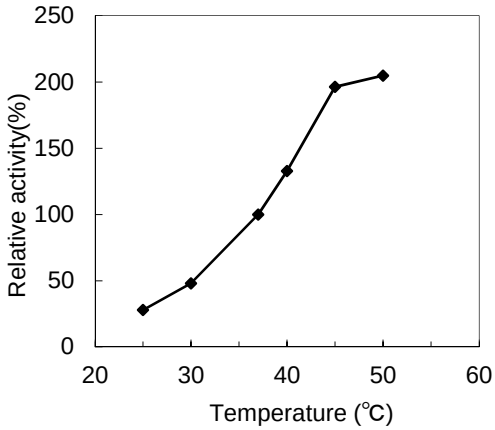
ΔA/min : The change in absorbance at 340 nm/minute (reverse the blank activation of NADS(-))
V : Total volume of reaction mixture (3.03 mL)
D : Enzyme dilution factor
6.3 : mmol/L extinction coefficient of NADH ($L \cdot mmol^{-1} \cdot cm^{-1}$)
d : Light path length (1 cm)
v : Volume of enzyme sample (0.02 mL)

Reference Data

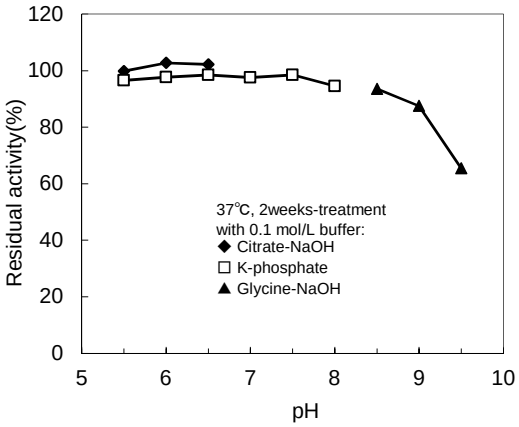
pH activity



Thermal activity



pH stability



Thermal stability