ASSAY

Principle



The fornation of NADH is measured at 340 nm by spectrophotometry.

Unit definition

One unit causes the formation of one micromole of NADH per minute under the conditions detailed below.

Method

A.Carbonate-bicarbonate buffer, pH 11.0	0.2 M (Prepare by mixing 0.2 M K2CO3 and 0.2 M NaHCO3 to reach pH 11.0).
B. Glycerol solution	0.3 M
C. Ammonium sulfate solution	1.0 M
D. NAD^+ solution	10 mM: Weigh out 143.5 mg of NAD＋ (MW ＝ 717.45), dissolve in 18.0 mL of H2O, and, after adjusting the pH to 7.0 with 0.5 N KOH, make up to 20.0 mL with H2O (should be freshly prepared).
E. Enzyme diluent	20 mM Potassium phosphate buffer pH 7.5.

Reagent

30.0 mL	Carbonate - bicarbonate buffer, pH 11.0	(A)
22.0 mL	Substrate solution	(B)
2.0 mL	Ammonium sulfate solution	(C)
6.0 mL	NAD^+ solution	(D)

Procedure

1.Prepare the following working solution, immediately before use.

Concentration in assay mixture	
Carbonate buffer	0.10 M
Glycero	0.10 M
NAD^+	1.0 mM
Ammonium sulfate	33 mM

1.Be sure the pH is in the range 10.0 to 10.5. If it is not, adjust to 10.5 with 1.0 N KOH or 1.0N HCl, and store on ice in a brownish bottle.

2.Pipette 2.9 mL of the working solution into a cuvette (d ＝ 1.0cm) and equilibrate at 25 ℃ for approximately 5 minutes.

3.Add 0.1 mL of the enzyme solution* and mix by gentle inversion.

4.Record the increase in optical density at 340 nm against water for 3 to 4 minutes with a spectrophotometer thermostated at 25 ℃ 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 enzyme solution.*Dissolve the enzyme preparation in ice-cold enzyme diluent (E), dilute to 0.10 − 0.25 U/mL with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/mL)} = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{6.22 \times 1.0 \times Vs}$$

$$= \Delta OD/min \times 4.82 \times df$$

$$\text{Weight activity (U/mg)} = (U/mL) \times 1/C$$

Vt : Total volume (3.0 mL)
Vs : Sample volume (0.1 mL)
6.22 : Millimolar extinction coefficient of NADH (cm^2/micromole)
1.0 : Light path length (cm)
df : Dilution factor
C : Enzyme concentration in dissolution (c mg/mL)