Enzymatic Assay of NADH PEROXIDASE
(EC 1.11.1.1)

PRINCIPLE:

ß-NADH + H_2O_2 $\xrightarrow{\text{NADH Peroxidase}}$ ß-NAD + $2H_2O$

Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH 5.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Acetate Buffer with 0.30 mM Ethylenediaminetetraacetic Acid, pH 5.4 at 25°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 5.4 at 25°C with either 1 M HCl or 1 M NaOH.)

B. 0.11% (v/v) Hydrogen Peroxide Solution (H_2O_2)
(Prepare 100 ml in deionized water using Hydrogen Peroxide, 30% (w/w) Solution, Sigma Prod. No. H-1009.)

C. 1000 mM Tris Solution (Tris)
(Prepare 10 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503.)

D. 23.4 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (ß-NADH)
(Dissolve the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Sodium Salt, Sigma Stock No. 340-110 in the appropriate volume of deionized water. Add one drop of Reagent C (Tris) per ml in order to neutralize the solution.)

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REAGENTS: (continued)

E. NADH Peroxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of NADH Peroxidase in cold deionized water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	2.57	2.57
Reagent B (H_2O_2)	0.36	0.36
Deionized Water	------	0.10

Mix by inversion and equilibrate to 25°C. Then add:

Reagent D (ß-NADH)	0.02	0.02
Reagent E (Enzyme Solution)	0.10	------

Immediately mix by inversion and monitor the decrease in A_{340nm} for approximately 5 minutes.

Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{\Delta A_{340}/\text{min Test} - \Delta A_{340nm}/\text{min Blank}}{(6.22)\ (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm.
RM = Reaction Mix

UNIT DEFINITION:

One unit will catalyze the oxidation of 1.0 µmole of ß-NADH per min at pH 5.4 at 25°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.05 ml reaction mix, the final concentrations are 84.3 mM sodium acetate, 0.25 mM ethylenediaminetetraacetic acid, 0.013% (v/v) hydrogen peroxide, 0.2 mM NADH, and 0.015 - 0.030 unit NADH peroxidase.

REFERENCE:

Dolin, M.I. (1957) *Journal of Biological Chemistry* **225**, 557 - 573.

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.