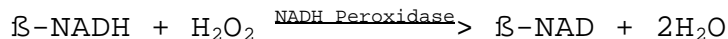


**Enzymatic Assay of NADH PEROXIDASE  
(EC 1.11.1.1)**

**PRINCIPLE:**



Abbreviations used:

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 25°C, pH 5.4, A<sub>340nm</sub>, 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<sub>2</sub>O<sub>2</sub>) (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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Solution ( $\beta$ -NADH) (Dissolve the contents of one 10 mg vial of  $\beta$ -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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.57	2.57
Reagent B (H <sub>2</sub> O <sub>2</sub> )	0.36	0.36
Deionized Water	-----	0.10

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

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

Immediately mix by inversion and monitor the decrease in  
A<sub>340nm</sub> for approximately 5 minutes.

Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for  
both the Test and Blank.

**CALCULATIONS:**

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

6.22 = Millimolar extinction coefficient of β-NADH at 340  
nm.

RM = Reaction Mix

**UNIT DEFINITION:**

One unit will catalyze the oxidation of 1.0 μmole of β-  
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.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**