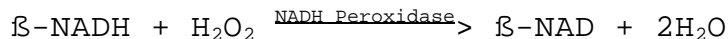


**Enzymatic Assay of NADH PEROXIDASE
(EC 1.11.1.1)**

PRINCIPLE:



Abbreviations used:

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH 6.0, A_{365nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Tris Acetate Buffer, pH 6.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Acetate, Sigma Prod. No. T-1258. Adjust to pH 6.0 at 25°C with 1 M HCl.)
- B. 0.30% (w/w) Hydrogen Peroxide Solution (H₂O₂)
(Prepare 10 ml in deionized water using Hydrogen Peroxide 30% (w/w) Solution, Sigma Prod. No. H-1009.)
- C. 12 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (NADH)
(Dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide, Reduced Form, Sodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water.)
- D. NADH Peroxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit of NADH Peroxidase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	3.00	3.00
Reagent C (NADH)	0.10	0.10
Reagent D (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Mix by inversion and equilibrate to 25°C. Monitor the baseline at $A_{365\text{nm}}$ for 5 minutes in order to determine any NADH oxidase activity which may be present.¹ Then add:

Reagent B (H ₂ O ₂)	0.10	0.10
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Immediately mix by inversion and monitor the decrease in $A_{365\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{365\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank using a suitably thermostatted spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{365\text{nm}}/\text{min Test} - r A_{365\text{nm}}/\text{min Blank})(3.3)(\text{df})}{(3.4)(0.1)}$$

3.3 = Total volume (in milliliters) of assay

df = Dilution factor

3.4 = Millimolar extinction coefficient² of β -NADH at 365 nm

0.1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will decompose 1.0 μmole of H₂O₂ per minute at pH 6.0 at 25°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.30 ml reaction mix, the final concentrations are 182 mM tris acetate, 0.009% (w/w) hydrogen peroxide, 0.36 mM β -nicotinamide adenine dinucleotide, and 0.02 - 0.04 unit NADH peroxidase.

REFERENCE:

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

Netheler, H.G. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume I, 184-187, Academic Press, Inc., New York, NY

NOTES:

1. The $A_{365\text{nm}}$ of both the Test and Blank must be corrected for the presence of any NADH oxidase activity.
2. Millimolar extinction coefficient of β -NADH at 365 nm as described in Netheler, H.G. (1974).
3. This assay is based on the cited reference, Dolin, M.I. (1957).
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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