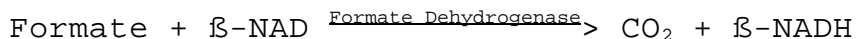


**Enzymatic Assay of FORMATE DEHYDROGENASE
(EC 1.2.1.2)
from Candida boidinii**

PRINCIPLE:



Abbreviations used:

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

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

CONDITIONS: T = 37°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Sodium Phosphate Buffer, pH 7.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust the pH to 7.5 with 1 M NaOH.)
- B. 200 mM Sodium Formate Solution (Form)
(Prepare 10 ml in deionized water using Formic Acid, Sodium Salt, Sigma Prod. No. F-6502. **PREPARE FRESH.**)
- C. 10.6 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Prepare 3 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004 or dissolve the contents of one 20 mg vial of β -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- D. 1.5 mM β -Nicotinamide Adenine Dinucleotide Solution (Enzyme Diluent)
(Prepare 50 ml in Reagent A using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)

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REAGENTS: (continued)

E. Formate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing
0.25 - 0.50 unit/ml of Formate Dehydrogenase in Cold
Reagent D.)

PROCEDURE:

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

	Test	Blank
Deionized Water	1.10	1.10
Reagent A (Buffer)	0.75	0.75
Reagent B (Form)	0.75	0.75

Mix by inversion and equilibrate to 37°C. Monitor the
A_{340nm} until constant, using a suitably thermostatted
spectrophotometer. Then add:

Reagent C (β-NAD)	0.30	0.30
Reagent D (Enzyme Diluent)	-----	0.10
Reagent E (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in
A_{340nm} for approximately 5 minutes. Obtain the r A_{340nm}/minute
using the maximum linear rate for both the Test and Blank.¹

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will oxidize 1.0 μ mole of formate to CO₂ per minute in the presence of β -NAD, at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 57 mM sodium phosphate, 50 mM formate, 1.1 mM β -nicotinamide adenine dinucleotide and 0.025 - 0.050 unit formate dehydrogenase.

REFERENCE:

Höpner, T. and Knappe, J. (1974) in *Methods of Enzymatic Analysis*, (Bergmeyer, H.U. ed.) Volume III, 1551-1555, Academic Press Inc., New York, NY

NOTES:

1. The linearity of the assay may be increased by increasing the β -NAD concentration 10 fold.
2. This assay is based on the cited reference.
3. 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.