Enzymatic Assay of FORMATE DEHYDROGENASE
(EC 1.2.1.2)

PRINCIPLE:

Formate + ß-NAD $\xrightarrow{\text{Formate Dehydrogenase}}$ CO_2 + ß-NADH

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

CONDITIONS: T = 37°C, pH = 7.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Sodium Phosphate Buffer, pH 7.0 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust the pH to 7.0 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. 10.5 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 3 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004 or dissolve the contents of one 20 mg vial of ß-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120, in the appropriate volume of deionized water. **PREPARE FRESH.**)

D. 1.5 mM ß-Nicotinamide Adenine Dinucleotide Solution (Enzyme Diluent)
(Prepare 50 ml in Reagent A using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH**.)

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.)

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

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of formate to CO_2 per minute in the presence of ß-NAD, at pH 7.0 at 37°C.

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FINAL ASSAY CONCENTRATION:

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

REFERENCE:

Hopner, T. and Knappe, J. (1974) *Methods of Enzymatic Analysis*, Volume III, 1551-1555

NOTES:

1. The linearity of the assay may be increased by increasing the ß-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.