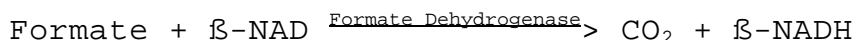


**Enzymatic Assay of FORMATE DEHYDROGENASE
(EC 1.2.1.2)**

PRINCIPLE:



Abbreviations used:

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

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

CONDITIONS: T = 37°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Monobasic Solution (KH₂PO₄)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- B. 100 mM Potassium Phosphate Dibasic Solution (K₂HPO₄)
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504.)
- C. 100 mM Potassium Phosphate Buffer, pH 7.6 at 37°C
(Prepare 100 ml by adjusting the pH of Reagent B to 7.6 at 37°C with Reagent A.)
- D. 1.0 M Sodium Formate Solution (Form)
(Prepare 10 ml in deionized water using Formic Acid, Sodium Salt, Sigma Prod. No. F-6502. **PREPARE FRESH.**)
- E. 60 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Prepare 3 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Lithium Salt, Sigma Prod. No. N-7132. **PREPARE FRESH.**)
- F. Formate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.45 - 0.90 unit/ml of Formate Dehydrogenase 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 C (Buffer)	2.50	2.50
Reagent D (Form)	0.50	0.50
Reagent E (β-NAD)	0.10	0.10

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

Reagent F (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3.2)(\text{df})}{(6.22)(0.1)}$$

3.2 = 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

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of formate to CO₂ per minute in the presence of β-NAD, at pH 7.6 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 3.20 ml reaction mix, the final concentrations are 78 mM potassium phosphate, 156 mM formate, 1.9 mM β -nicotinamide adenine dinucleotide and 0.045 - 0.090 unit formate dehydrogenase.

REFERENCE:

Höpner, T. and Knappe, J. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume III, 1551-1555

NOTES:

1. This assay is based on the cited reference.
2. 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.