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 = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Monobasic Solution (KH_2PO_4)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)

B. 100 mM Potassium Phosphate Dibasic Solution (K_2HPO_4)
(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 ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 3 ml in deionized water using ß-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:

	Test	Blank
Reagent C (Buffer)	2.50	2.50
Reagent D (Form)	0.50	0.50
Reagent E (ß-NAD)	0.10	0.10

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

	Test	Blank
Reagent F (Enzyme Solution)	0.10	------
Deionized Water	------	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.

CALCULATIONS:

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

3.2 = Total Volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of 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 7.6 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 ß-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.