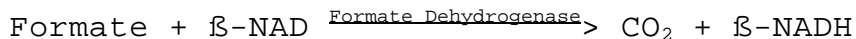


**Enzymatic Assay of FORMATE DEHYDROGENASE  
(EC 1.2.1.2)**

**PRINCIPLE:**



Abbreviations used:

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

**CONDITIONS:** T = 37°C, pH = 7.0, A<sub>340nm</sub>, 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  $\beta$ -Nicotinamide Adenine Dinucleotide Solution ( $\beta$ -NAD)  
(Prepare 3 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004 or dissolve the contents of one 20 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- D. 1.5 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Solution (Enzyme Diluent)  
(Prepare 50 ml in Reagent A using  $\beta$ -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:

	<u>Test</u>	<u>Blank</u>
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  $A_{340\text{nm}}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C ( $\beta$ -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  $A_{340\text{nm}}$  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:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu\text{mole}$  of formate to  $\text{CO}_2$  per minute in the presence of  $\beta$ -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  $\beta$ -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  $\beta$ -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.**