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

**PRINCIPLE:**

Formate + β-NAD $\xrightarrow{\text{Formate Dehydrogenase}}$ CO$_2$ + β-NADH

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

**CONDITIONS:** $T = 37^\circ C$, 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.  
   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.  
   PREPARE FRESH.)

C. 10.6 mM β-Nicotinamide Adenine Dinucleotide Solution (β-NAD)  
   (Prepare 3 ml in deionized water using β-Nicotinamide Adenine Dinucleotide or  
   dissolve the contents of one 20 mg vial of β-Nicotinamide Adenine Dinucleotide,  
   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.  
   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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>1.10</td>
<td>1.10</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Reagent B (Form)</td>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (β-NAD)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent D (Enzyme Diluent)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

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 μmole 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:


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.