Enzymatic Assay of FORMATE DEHYDROGENASE
(EC 1.2.1.2)

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: $T = 37^\circ\text{C}$, pH = 7.6, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Monobasic Solution (KH$_2$PO$_4$)
   (Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)

B. 100 mM Potassium Phosphate Dibasic Solution (K$_2$HPO$_4$)
   (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 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Buffer)</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent D (Form)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent E (β-NAD)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the A₃₄₀nm 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 F (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A₃₄₀nm for approximately 5 minutes. Obtain the r A₃₄₀nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{\text{A}_{340\text{nm}}/\text{min Test}} - r_{\text{A}_{340\text{nm}}/\text{min Blank}})(3.2)(df)}{(6.22)(0.1)}
\]

- 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:


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.