Enzymatic Assay of FORMALDEHYDE DEHYDROGENASE  
(EC 1.2.1.46)

PRINCIPLE:

Formaldehyde + β-NAD $\xrightarrow{FDH}$ Formate + β-NADH

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

CONDITIONS:  T = 37°C, pH = 7.5, $A_{340\text{nm}}$, Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 37°C  
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous.  
Adjust to pH 7.5 at 37°C with 1 M KOH.)

B. 5.7 mM β-Nicotinamide Adenine Dinucleotide Solution  
(β-NAD) 
(Prepare 5 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.)

C. 0.08% (v/v) Formaldehyde Solution (Formaldehyde)  
(Prepare by adding 0.1 ml of Formaldehyde, 37% (w/w)  
Solution (Formalin), to 45 ml of deionized water.)

D. Formaldehyde Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing  
0.5 – 1.0 unit/ml of Formaldehyde Dehydrogenase in  
cold Reagent A.)
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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>
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</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.00</td>
<td>2.05</td>
</tr>
<tr>
<td>Reagent B (β-NAD)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent C (Formaldehyde)</td>
<td>0.10</td>
<td>0.10</td>
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</tbody>
</table>

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

Reagent D (Enzyme Solution) 0.05 -------

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:

\[
\text{Units/ml enzyme} = \frac{(r \ A_{340nm}/\text{min Test} - r \ A_{340nm}/\text{min Blank})(2.95)(df)}{(6.22)(0.05)}
\]

2.95 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.05 = Volume (in milliliter) of enzyme used

Unit Definition:

One unit will oxidize 1.0 µmole of formaldehyde to formic acid per minute at pH 7.5 at 37°C.
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FINAL ASSAY CONCENTRATION:

In a 2.95 ml reaction mix, the final concentrations are 35 mM potassium phosphate, 1.0 mM β-nicotinamide adenine dinucleotide, 0.003% (v/v) formaldehyde, and 0.03 - 0.05 unit formaldehyde dehydrogenase.

REFERENCES:


NOTES:

1. This assay is based on the cited reference.

2. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.