**Enzymatic Assay of MYO-INOSITOL DEHYDROGENASE**  
(EC 1.1.1.18)

**PRINCIPLE:**

\[
\text{myo-Inositol} + \beta\text{-NAD} \xrightarrow{\text{myo-Inositol Dehydrogenase}} \text{scyllo- } \text{Inosose} + \beta\text{-NADH}
\]

**Abbreviations:**  
\(\beta\text{-NAD} = \beta\text{-Nicotinamide Adenine Dinucleotide}\)  
\(\beta\text{-NADH} = \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form}\)

**CONDITIONS:**  
\(T = 25^\circ C, \text{pH } = 9.0, A_{340nm}, \text{Light path } = 1 \text{ cm}\)

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Sodium Pyrophosphate Buffer, pH 9.0 at 25°C  
(Prepare 100 ml in deionized water using Tetrasodium Pyrophosphate, Anhydrous.  
Adjust to pH 9.0 at 25°C with 1 M HCl.)

B. 5 mM \(\beta\text{-Nicotinamide Adenine Dinucleotide Solution (}\beta\text{-NAD)}\)  
(Prepare 5 ml in deionized water using \(\beta\text{-Nicotinamide Adenine Nucleotide. Adjust to pH 7.0 at 25C with solid Sodium Bicarbonate.)}\)

C. 250 mM myo-Inositol Solution (Substrate)  
(Prepare 5 ml in deionized water using myo-Inositol.)

D. 20 mM Potassium Phosphate Buffer, pH 7.0 at 25°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic. Adjust to pH 7.0 at 25C with 1 M NaOH.)

E. myo-Inositol Dehydrogenase Solution  
(Immediately before use, prepare a solution containing 4 units/ml of myo-Inositol Dehydrogenase in cold
Reagent D.)
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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 A (Buffer)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent B (β-NAD)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Reagent C (Substrate)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>2.10</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A$_{340\text{nm}}$ 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 A (Buffer)</td>
<td>------</td>
<td>0.01</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.01</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A$_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r$ A$_{340\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r \text{ A}_{340\text{nm}}/\text{min Test} - r \text{ A}_{340\text{nm}}/\text{min Blank})(3.01)(df)}{(6.22)(0.01)}
\]

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

Units/mg solid = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}

Units/mg protein = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}

UNIT DEFINITION:

One unit will convert 1.0 µmole of myo-inositol and β-NAD to scyllo-inosose and β-NADH per minute at pH 9.0 at 25°C.
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FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 10 mM sodium pyrophosphate, 0.5 mM β-nicotinamide adenine dinucleotide, 25 mM myo-inositol and 0.04 unit myo-inositol 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.