**Enzymatic Assay of MANNITOL DEHYDROGENASE**  
**(EC 1.1.1.67)**

**PRINCIPAL:**

\( \text{D(-)Fructose + ß-NADH} \xrightarrow{\text{Mannitol Dehydrogenase}} \text{D-Mannitol + ß-NAD} \)

Abbreviations used:

\( ß\text{-NADH} = ß\text{-Nicotinamide Adenine Dinucleotide, Reduced Form} \)

\( ß\text{-NAD} = ß\text{-Nicotinamide Adenine Dinucleotide, Oxidized Form} \)

**CONDITIONS:**  \( T = 30^\circ C, \ pH = 5.3, \ A_{340nm}, \) Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 50 mM Sodium Acetate Buffer, pH 5.3 at 30°C  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate. Adjust to pH 5.3 at 30°C with Acetic Acid, Glacial.)

B. 1000 mM D(-)Fructose Solution (Fruc)  
(Prepare 10 ml in deionized water using \text{D(-)Fructose.})

C. 10 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)  
(Prepare 1 ml in cold deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt. **PREPARE FRESH**)

D. Mannitol Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.1 - 0.5 unit/ml of Mannitol 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>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.630</td>
<td>0.630</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.400</td>
<td>0.400</td>
</tr>
<tr>
<td>Reagent C (β-NADH)</td>
<td>0.025</td>
<td>0.025</td>
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</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
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<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.120</td>
</tr>
<tr>
<td>Reagent B (Fruc)</td>
<td>0.120</td>
<td>------</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.025</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease 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:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(1.2)(df)}{(6.22)(0.025)}$$

1.2 = Total volume (in milliliters) of assay  
$df$ = Dilution factor  
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm  
0.025 = 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 reduce 1.0 µmole of ρ(-)-fructose per minute in the presence of β-NADH at pH 5.3 at 30°C.
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FINAL ASSAY CONCENTRATION:

In a 1.20 ml reaction mix, the final concentrations are 18 mM sodium acetate, 100 mM D(-)fructose, 0.21 mM β-nicotinamide adenine dinucleotide, 0.0025 - 0.0125 unit mannitol dehydrogenase.

REFERENCE:


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.