Enzymatic Assay of SORBITOL DEHYDROGENASE  
(EC 1.1.1.14)

PRINCIPLE:

\[
\text{Sorbitol Dehydrogenase} \\
\text{d-Fructose + } \beta\text{-NADH} \rightarrow \text{d-Sorbitol + } \beta\text{-NAD}
\]

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

CONDITIONS:  \( T = 25^\circ\text{C}, \; \text{pH} 7.6, \; A_{340\text{nm}}, \; \text{Light path} = 1 \; \text{cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Triethanolamine Buffer, pH 7.6 at 25°C  
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 5 M NaOH.)

B. 1.1 M d-Fructose Solution (Fructose)  
(Prepare 5 ml in deionized water using d(-)-Fructose, Sigma Prod. No. F-0127.)

C. 12.8 mM \( \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (}\beta\text{-NADH)} \)  
(Dissolve the contents of one 10 mg vial of \( \beta\text{-Nicotinamide Adenine Dinucleotide, Reduced Form, Preweighed Vial, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. PREPARE FRESH.)

D. 1.0% (w/v) Bovine Serum Albumin (BSA)  
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-6003.)

E. Sorbitol Dehydrogenase Enzyme Solution  
(Prepare a solution containing 70 - 150 units/ml of Sorbitol Dehydrogenase in cold deionized water. Store at 4°C for 1 hour. Immediately before use, dilute to a final concentration of 0.55 - 0.75 unit/ml with 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>2.35</td>
<td>2.35</td>
</tr>
<tr>
<td>Reagent B (Fructose)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent C ((\beta)-NADH)</td>
<td>0.05</td>
<td>0.05</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 E (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent D (BSA)</td>
<td>------</td>
<td>0.10</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:

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

- \(3\) = Total volume (in milliliters) of assay  
- \(\text{df}\) = Dilution factor  
- 6.22 = Millimolar extinction coefficient of \(\beta\)-NADH at 340nm  
- 0.1 = Volume (in milliliter) of enzyme

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

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

UNIT DEFINITION:

One unit will convert 1.0 \(\mu\)mole of \(\alpha\)-fructose to \(\alpha\)-sorbitol per minute at pH 7.6 at 25°C.
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 78 mM triethanolamine, 183 mM D-fructose, 0.2 mM β-nicotinamide adenine dinucleotide, reduced form, 0.033% (w/v) bovine serum albumin and 0.055 - 0.075 unit sorbitol 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.