Enzymatic Assay of POLYOL DEHYDROGENASE  
(EC 1.1.1.14)

PRINCIPLE:
Xylitol + β-NAD → Polyol Dehydrogenase → D-Xylulose + β-NADH

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

CONDITIONS:  T = 25°C, pH = 8.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Glycine Buffer, pH 8.6 at 25°C  
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 8.6 at 25°C with 1 M NaOH.)

B. 2.4 M Xylitol Solution (Xylitol)  
(Prepare 3 ml in deionized water using Xylitol, Sigma Prod. No. X-3375. PREPARE FRESH.)

C. 186 mM β-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (β-NAD)  
(Prepare 2 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Sodium Salt, Sigma Prod. No. N-0632.)

D. 10 mM 2-Mercaptoethanol Solution (2-ME)  
(Prepare 4 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

E. Polyol Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Polyol Dehydrogenase in cold deionized water.)
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PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Reagent A (Buffer)</th>
<th>23.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Xylitol)</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent C (β-NAD)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent D (2-ME)</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Mix by swirling and adjust to pH 8.6 at 25°C with 1 M NaOH.

Pipette (in milliliters) the following reagents into suitable cuvettes:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.02</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A<sub>340nm</sub> for approximately 5 minutes. Obtain the r<sub>340nm/min</sub> using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}})(2.92)(df)}{(6.22)(0.02)}
\]

2.92 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADH at 340nm

0.02 = Volume (in milliliter) of enzyme used

\[
\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}
\]
Units/mg protein = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
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UNIT DEFINITION:

One unit will convert 1.0 μmole of xylitol to D-xylulose per minute at pH 8.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 2.92 ml reaction mix, the final concentrations are 77 mM glycine, 202 mM xylitol, 6.3 mM β-nicotinamide adenine dinucleotide, 1 mM 2-mercaptoethanol, and 0.02 - 0.04 unit polyol dehydrogenase.

REFERENCES:


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.