Enzymatic Assay of POLYOL DEHYDROGENASE
(EC 1.1.1.14)

PRINCIPLE:
Xylitol + ß-NAD $\xrightarrow{\text{Polyol Dehydrogenase}}$ D-Xylulose + ß-NADH

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-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. 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. PREPARE FRESH.)

C. 186 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (ß-NAD)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sodium Salt.)

D. 10 mM 2-Mercaptoethanol Solution (2-ME)
(Prepare 4 ml in deionized water using 2-Mercaptoethanol.)

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:

- Reagent A (Buffer)  23.00
- Reagent B (Xylitol)  2.50
- Reagent C (β-NAD)  1.00
- Reagent D (2-ME)   3.00

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>Reaction Cocktail</th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2.90</td>
<td>2.90</td>
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Equilibrate to 25°C. Monitor the A\textsubscript{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

- Reagent E (Enzyme Solution)  0.02
- Deionized Water  ------

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

CALCULATIONS:

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

2.92 = Total volume (in milliliters) of assay
\( \text{df} \) = 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.

This procedure is for informational purposes.