Enzymatic Assay of L-XYLOLOSE REDUCTASE
(EC 1.1.1.10)

PRINCIPLE:

\[
\text{Xylitol} + \beta-\text{NADP} \xrightarrow{\text{Xylulose Reductase}} \text{L-Xylulose} + \beta-\text{NADPH}
\]

Abbreviations used:

\begin{align*}
\beta-\text{NADPH} &= \beta-\text{Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form} \\
\beta-\text{NADP} &= \beta-\text{Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form}
\end{align*}

CONDITIONS:  \( T = 25^\circ\text{C}, \, \text{pH} = 10.0, \, A_{340nm}, \, \text{Light path} = 1 \text{ cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  100 mM Glycine Buffer, pH 10.0 at 25°C
   (Prepare 100 ml in deionized water using Glycine, Free Base. Adjust to pH 10.0 at 25°C with 1 M NaOH.)

B.  100 mM Magnesium Chloride Solution (\(\text{MgCl}_2\))
   (Prepare 5 ml in deionized water using Magnesium Chloride, 4.9 M Solution.)

C.  657 mM Xylitol Solution (Xylitol)
   (Prepare 5 ml in deionized water using Xylitol)

D.  12.5 mM \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate Solution (\(\beta\)-NADP)
   (Prepare 2 ml in deionized water using \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt)

E.  L-Xylulose Reductase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of L-Xylulose Reductase 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>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent B (MgCl$_2$)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (Xylitol)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent D (β-NADP)</td>
<td>0.10</td>
<td>0.10</td>
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Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<p>| | | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.10</td>
<td>-------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
<td>0.10</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:

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

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of xylitol to L-xylulose per minute at pH 10.0 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 87 mM glycine, 3.3 mM magnesium chloride, 44 mM xylitol, 0.42 mM β-nicotinamide adenine dinucleotide phosphate, and
0.01 - 0.02 unit l-xylulose reductase.
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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.