Enzymatic Assay of L-XYLULOSE REDUCTASE
(EC 1.1.1.10)

PRINCIPLE:

Xylitol + ß-NADP $\xrightarrow{\text{Xylulose Reductase}}$ L-Xylulose + ß-NADPH

Abbreviations used:
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

CONDITIONS: T = 25°C, pH = 10.0, A_{340nm}, Light path = 1 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, Sigma Prod. No. G-7126. Adjust to pH 10.0 at 25°C with 1 M NaOH.)

B. 100 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 5 ml in deionized water using Magnesium Chloride, 4.9 M Solution, Sigma Stock No. 104-20.)

C. 657 mM Xylitol Solution (Xylitol)
(Prepare 5 ml in deionized water using Xylitol, Sigma Prod. No. X-3375.)

D. 12.5 mM ß-Nicotinamide Adenine Dinucleotide Phosphate Solution (ß-NADP)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)

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:

	Test	Blank
Reagent A (Buffer)	2.50	2.50
Reagent B ($MgCl_2$)	0.10	0.10
Reagent C (Xylitol)	0.20	0.20
Reagent D (ß-NADP)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.10	------
Reagent A (Buffer)	------	0.10

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-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 ß-nicotinamide adenine dinucleotide phosphate, and

0.01 - 0.02 unit L-xylulose reductase.

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REFERENCES:

Touster, O. and Montesi, G. (1962) *Methods in Enzymology*, V, 317-322

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.