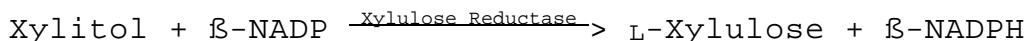


**Enzymatic Assay of L-XYLULOSE REDUCTASE  
(EC 1.1.1.10)**

**PRINCIPLE:**



Abbreviations used:

$\beta$ -NADPH =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

$\beta$ -NADP =  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

**CONDITIONS:** T = 25°C, pH = 10.0,  $A_{340\text{nm}}$ , 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 ( $\text{MgCl}_2$ )  
(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  $\beta$ -Nicotinamide Adenine Dinucleotide Phosphate Solution ( $\beta$ -NADP)  
(Prepare 2 ml in deionized water using  $\beta$ -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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.50	2.50
Reagent B (MgCl <sub>2</sub> )	0.10	0.10
Reagent C (Xylitol)	0.20	0.20
Reagent D (β-NADP)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> 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<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/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:**

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.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**