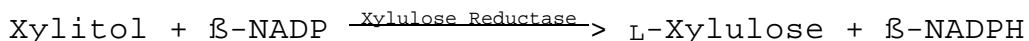


**Enzymatic Assay of L-XYLULOSE REDUCTASE
(EC 1.1.1.10)**

PRINCIPLE:



Abbreviations used:

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

β -NADP = β -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 (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 β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Prepare 2 ml in deionized water using β -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 ₂) | 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_{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 r A_{340nm}/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.