

**Enzymatic Assay of POLYOL DEHYDROGENASE
(EC 1.1.1.14)**

PRINCIPLE:

Xylitol + β -NAD $\xrightarrow{\text{Polyol Dehydrogenase}}$ D-Xylulose + β -NADH

Abbreviations used:

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

β -NADH = β -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, Sigma Prod. No. G-7126. 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, Sigma Prod. No. X-3375. **PREPARE FRESH.**)
- C. 186 mM β -Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (β -NAD)
(Prepare 2 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sodium Salt, Sigma Prod. No. N-0632.)
- D. 10 mM 2-Mercaptoethanol Solution (2-ME)
(Prepare 4 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250.)
- 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:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.90	2.90

Equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r_{A_{340nm}/\text{min Test}} - r_{A_{340nm}/\text{min Blank}})(2.92)(df)}{(6.22)(0.02)}$$

2.92 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADH at 340nm

0.02 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will convert 1.0 μ mole 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:

McCorkindale, J. and Edson, N.L. (1954) *Biochemical Journal* **57**, 518-523

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.