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. 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.)

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

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
)

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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