Enzymatic Assay of SORBITOL DEHYDROGENASE (EC 1.1.1.14)

PRINCIPLE:

$$\text{D-Fructose} + \text{ß-NADH} \xrightarrow{\text{Sorbitol Dehydrogenase}} \text{D-Sorbitol} + \text{ß-NAD}$$

Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Triethanolamine Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 5 M NaOH.)

B. 1.1 M D-Fructose Solution (Fructose)
(Prepare 5 ml in deionized water using D(-)Fructose, Sigma Prod. No. F-0127.)

C. 12.8 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (ß-NADH)
(Dissolve the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Preweighed Vial, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH**.)

D. 1.0% (w/v) Bovine Serum Albumin (BSA)
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-6003.)

E. Sorbitol Dehydrogenase Enzyme Solution
(Prepare a solution containing 70 - 150 units/ml of Sorbitol Dehydrogenase in cold deionized water. Store at 4°C for 1 hour. Immediately before use, dilute to a final concentration of 0.55 - 0.75 unit/ml with cold Reagent D.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	2.35	2.35
Reagent B (Fructose)	0.50	0.50
Reagent C (ß-NADH)	0.05	0.05

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 D (BSA)	------	0.10

Immediately mix by inversion and record the decrease 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-NADH at 340nm
0.1 = Volume (in milliliter) of enzyme

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of D-fructose to D-sorbitol per minute at pH 7.6 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 78 mM triethanolamine, 183 mM D-fructose, 0.2 mM ß-nicotinamide adenine dinucleotide, reduced form, 0.033% (w/v) bovine serum albumin and 0.055 - 0.075 unit sorbitol dehydrogenase.

REFERENCE:

Gerlach, U. and Hiby, W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume II, 569-573, Academic Press Inc., New York, NY

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.