Enzymatic Assay of MANNITOL DEHYDROGENASE
(EC 1.1.1.67)

PRINCIPAL:

$_{D}$(-)Fructose + ß-NADH $\xrightarrow{\text{Mannitol Dehydrogenase}}$ $_{D}$-Mannitol + ß-NAD

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

CONDITIONS: T = 30°C, pH = 5.3, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Acetate Buffer, pH 5.3 at 30°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.3 at 30°C with Acetic Acid, Glacial, Sigma Prod. No. A-6283.)

B. 1000 mM $_{D}$(-)Fructose Solution (Fruc)
(Prepare 10 ml in deionized water using $_{D}$(-)Fructose, Sigma Prod. No. F-0127.)

C. 10 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)
(Prepare 1 ml in cold deionized water using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH**.)

D. Mannitol Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.5 unit/ml of Mannitol Dehydrogenase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Deionized Water	0.630	0.630
Reagent A (Buffer)	0.400	0.400
Reagent C (ß-NADH)	0.025	0.025

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized Water	------	0.120
Reagent B (Fruc)	0.120	------
Reagent D (Enzyme Solution)	0.025	0.025

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:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(1.2)(df)}{(6.22)(0.025)}$$

1.2 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm 0.025 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will reduce 1.0 µmole of D(-)fructose per minute in the presence of ß-NADH at pH 5.3 at 30°C.

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FINAL ASSAY CONCENTRATION:

In a 1.20 ml reaction mix, the final concentrations are 18 mM sodium acetate, 100 mM D(-)fructose, 0.21 mM ß-nicotinamide adenine dinculeotide, 0.0025 - 0.0125 unit mannitol dehydrogenase.

REFERENCE:

Yamanaka, K. (1975) *Methods in Enzymology 41*, Part B, 138-142

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.