

**Enzymatic Assay of MANNITOL DEHYDROGENASE**  
**(EC 1.1.1.67)**  
**From Actinobacillus sp.**

**PRINCIPLE:**

D-Mannitol +  $\beta$ -NAD Mannitol Dehydrogenase > D-Fructose +  $\beta$ -NADH

Abbreviations used:

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

**CONDITIONS:** T = 37°C, pH = 7.6, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 60 mM Potassium Phosphate Buffer, pH 7.6 at 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.6 at 37°C with 1 M KOH.)
- B. 500 mM Mannitol Solution (Mannitol)  
(Prepare 5 ml in deionized water using D-Mannitol, Sigma Prod. No. M-4125.)
- C. 5.0 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution ( $\beta$ -NAD)  
(Dissolve the contents of a 50 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150 in the appropriate volume of deionized water.)
- D. Mannitol Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.10 - 0.20 unit/ml of Mannitol Dehydrogenase 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)	1.00	1.00
Reagent B (Mannitol)	0.20	0.20
Reagent C (β-NAD)	1.00	1.00
Deionized Water	0.80	0.80

Mix by inversion and equilibrate to 37°C. Monitor the  $A_{340\text{nm}}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme solution)	0.10	-----
Reagent A (Buffer)	-----	0.10

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

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3.1)(\text{df})}{(6.22)(0.1)}$$

3.1 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

**UNIT DEFINITION:**

One unit will convert 1.0 μmole of D-mannitol to D-fructose per minute at pH 7.6 at 37°C.

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**FINAL ASSAY CONCENTRATION:**

In a 3.10 ml reaction mix, the final concentrations are 21 mM potassium phosphate, 32 mM mannitol, 1.6 mM  $\beta$ -nicotinamide adenine dinucleotide and 0.01 - 0.02 unit mannitol dehydrogenase.

**REFERENCE:**

Martinez, G., Barker, H.A. and Horecker, B.L. (1963)  
*Journal of Biological Chemistry*, 238, 1598-1603

**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.**