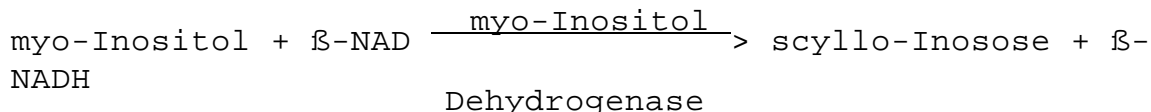


**Enzymatic Assay of MYO-INOSITOL DEHYDROGENASE  
(EC 1.1.1.18)**

**PRINCIPLE:**



Abbreviations:

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide

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

**CONDITIONS:** T = 25°C, pH = 9.0, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 100 mM Sodium Pyrophosphate Buffer, pH 9.0 at 25°C  
(Prepare 100 ml in deionized water using Tetrasodium Pyrophosphate, Anhydrous, Sigma Prod. No. P-8010. Adjust to pH 9.0 at 25°C with 1 M HCl.)
- B. 5 mM  $\beta$ -Nicotinamide Adenine Dinucleotide Solution ( $\beta$ -NAD)  
(Prepare 5 ml in deionized water using  $\beta$ -Nicotinamide Adenine Nucleotide, Sigma Prod. No. N-7004. Adjust to pH 7.0 at 25°C with solid Sodium Bicarbonate, Sigma Prod. No. S-6014.)
- C. 250 mM myo-Inositol Solution (Substrate)  
(Prepare 5 ml in deionized water using myo-Inositol, Sigma Prod. No. I-5125.)
- D. 20 mM Potassium Phosphate Buffer, pH 7.0 at 25°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 25°C with 1 M NaOH.)
- E. myo-Inositol Dehydrogenase Solution  
(Immediately before use, prepare a solution containing 4 units/ml of myo-Inositol Dehydrogenase in cold

Reagent D.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.30	0.30
Reagent B (β-NAD)	0.30	0.30
Reagent C (Substrate)	0.30	0.30
Deionized Water	2.10	2.10

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent A (Buffer)	-----	0.01
Reagent E (Enzyme Solution)	0.01	-----

Immediately mix by inversion and record the increase in A<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r_{A_{340nm}/\text{min Test}} - r_{A_{340nm}/\text{min Blank}})(3.01)(df)}{(6.22)(0.01)}$$

3.01 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.01 = 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 convert 1.0 μmole of myo-inositol and β-NAD to scyllo-inosose and β-NADH per minute at pH 9.0 at 25°C.

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**FINAL ASSAY CONCENTRATION:**

In a 3.01 ml reaction mix, the final concentrations are 10 mM sodium pyrophosphate, 0.5 mM  $\beta$ -nicotinamide adenine dinucleotide, 25 mM myo-inositol and 0.04 unit myo-inositol dehydrogenase.

**REFERENCES:**

Berman T. and Magasanik B. (1966) *Journal of Biological Chemistry* **241**, 800-806

**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.**