Enzymatic Assay of MYO-INOSITOL DEHYDROGENASE
(EC 1.1.1.18)

PRINCIPLE:

myo-Inositol + ß-NAD $\xrightarrow[\text{Dehydrogenase}]{\text{myo-Inositol}}$ scyllo-Inosose + ß-NADH

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

CONDITIONS: T = 25°C, pH = 9.0, A_{340nm}, 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 ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 5 ml in deionized water using ß-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:

	Test	Blank
Reagent A (Buffer)	0.30	0.30
Reagent B (ß-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_{340nm} 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_{340nm} for approximately 5 minutes. Obtain the $r\ A_{340nm}$/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 ß-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 ß-NAD to scyllo-inosose and ß-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 ß-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.