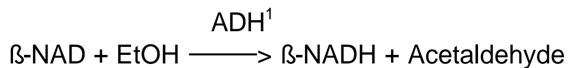
Enzymatic Assay of NADase
(EC 3.2.2.5)

PRINCIPLE:



Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ADP-ribose = Adenosine 5'-Diphosphate-Ribose
ADH = Alcohol Dehydrogenase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 37°C, pH = 7.3, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.3 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.3 at 37°C with 1 M NaOH.)

B. 7.5 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (ß-NAD)
(Prepare 5 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004.)

C. 3 M Trichloroacetic Acid Solution (TCA)
(Prepare 10 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)

D. 453 mM Glycine Buffer, pH 9.8 at 25°C
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 9.8 at 25°C with 1 M NaOH.)

REAGENTS: (continued)

E. 74 mM Pyrophosphate, 75 mM Semicarbazide and 1% (v/v) Ethanol (ADH React. Cocktail)
(Prepare 100 ml in Reagent D using Pyrophosphate, Tetrasodium, Decahydrate, Sigma Prod. No. P-9146, Semicarbazide Hydrochloride, Sigma Prod. No. S-4125, and 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)

F. Alcohol Dehydrogenase Enzyme Solution (ADH)
(Immediately before use, prepare a solution containing approximately 5000 units/ml of Alcohol Dehydrogenase, Sigma Prod. Nos. A-7011 or A-3263 in cold Reagent A.)

G. NADase Enzyme Solution (NADase)
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of NADase in cold Reagent A.)

PROCEDURE:

Step 1

Pipette (in milliliters) the following reagents into suitable centrifuge tubes:

	Test	Blank
Reagent A (Buffer)	0.80	0.80
Reagent B (ß-NAD)	0.20	0.20
Reagent C (TCA)	------	0.30

Mix by inversion and equilibrate to 37°C. Then add:

	Test	Blank
Reagent G (NADase)	0.20	0.20

Immediately mix by inversion and incubate for exactly 20 minutes at 37°C. Then add:

	Test	Blank
Reagent C (TCA)	0.30	------

Mix by inversion and centrifuge both the Test and Blank solutions.

PROCEDURE: (continued)

Step 2

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

	Test	Blank
Test Supernatant	0.40	------
Blank Supernatant	------	0.40
Reagent E (ADH React. Cocktail)	2.60	2.60

Mix by inversion and equilibrate to 25°C using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both the Test and Blank. Then add:

	Test	Blank
Reagent F (ADH)	0.01	0.01

Immediately mix by inversion and monitor the increase in A_{340nm} until constant. The maximum increase in A_{340nm} should be obtained after 5 - 10 minutes at 25°C. Record the final A_{340nm} for both the Test and Blank.

CALCULATIONS:

$$\Delta A_{340nm} = \text{Final } A_{340nm} - \text{Initial } A_{340nm}$$

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}\text{ Blank} - \Delta A_{340nm}\text{ Test})(1.5)(3.01)(df)}{(20)(6.22)(0.2)(0.4)}$$

1.5 = Total volume (in milliliters) of Step 1
3.01 = Total volume (in milliliters) of Step 2
df = Dilution factor
20 = Time (in minutes) of Step 1 assay as per the Unit Definition
6.22 = Millimolar extinction coefficient of ß-NADH at 340nm
0.2 = Volume (in milliliter) of enzyme used
0.4 = Volume (in milliliter) of Step 1 used in Step 2

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

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of ß-NAD to nicotinamide and ADP-ribose per minute at pH 7.3 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.20 ml reaction mix, the final concentrations are 83 mM potassium phosphate, 1.3 mM ß-nicotinamide adenine dinucleotide and 0.02 - 0.04 unit NADase.

REFERENCE:

Kaplan, N. O. (1955) *Methods in Enzymology*, Vol II, 660-663

NOTES:

1. The amount of ß-NAD consumed in the first reaction catalyzed by NADase is determined by measuring the residual ß-NAD using this reaction.
2. This assay is based on the cited reference.
3. 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.