SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of NADase
(EC 3.2.2.5)

PRINCIPLE:

$$\beta\text{-NAD} + H_2O \xrightarrow{\text{NADase}} \text{Nicotinamide} + \text{ADP-ribose}$$

$$\beta\text{-NAD} + \text{EtOH} \xrightarrow{\text{ADH}^1} \beta\text{-NADH} + \text{Acetaldehyde}$$

Abbreviations used:
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
ADP-ribose = Adenosine 5'-Diphosphate-Ribose
ADH = Alcohol Dehydrogenase
β-NADH = β-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 β-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (β-NAD)
(Prepare 5 ml in deionized water using β-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 (β-NAD)	0.20	0.20
Reagent C (TCA)	------	0.30

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

Reagent G (NADase)	0.20	0.20

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

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:

ΔA_{340nm} = Final A_{340nm} - 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 β-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 β-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 β-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 β-NAD consumed in the first reaction catalyzed by NADase is determined by measuring the residual β-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.