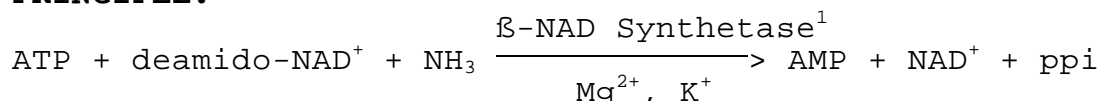


**Enzymatic Assay of β -NAD SYNTHETASE
(EC 6.3.5.1)**

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

Deamido-NAD⁺ = Nicotinic Acid Adenine Dinucleotide

AMP = Adenosine 5'-Monophosphate

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

ppi = Inorganic Pyrophosphate

CONDITIONS: T = 37°C, pH = 8.5, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 120 mM Tris HCl Buffer, pH 8.5 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.5 at 37°C with 1 M HCl.)
- B. 30 mM Nicotinic Acid Adenine Dinucleotide Solution (deamido-NAD⁺)
(Prepare 1 ml in deionized water using Nicotinic Acid Adenine Dinucleotide, Sigma Prod. No. N-4256.)
- C. 60 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-2383.)
- D. 30 mM Ammonium Sulfate Solution ((NH₄)₂SO₄)
(Prepare 1 ml in deionized water using Ammonium Sulfate, Sigma Prod. No. A-5132.)
- E. 1% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 1 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-7906.)

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REAGENTS: (continued)

- F. 240 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Anhydrous, Sigma Prod. No. M-8266.)
- G. 120 mM Potassium Chloride Solution (KCl)
(Prepare 10 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)
- H. Alcohol Dehydrogenase Enzyme Solution (ADH)
(Immediately before use, prepare a solution containing 200 units/ml of Alcohol Dehydrogenase, Sigma Prod. No. A-3263, in cold deionized water².)
- I. 95% (v/v) Ethanol (EtOH)
(Prepare 1 ml in deionized water using 200 Proof Ethyl Alcohol, available from Quantum Chemical Company.)
- J. β -NAD Synthetase Enzyme Solution (β -NAD Synth)
(Immediately before use, prepare a solution containing 0.15 - 1.0 unit/ml of β -NAD Synthetase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.55	1.55
Reagent B (deamido-NAD ⁺)	0.10	0.10
Reagent C (ATP)	0.10	0.10
Reagent D ((NH ₄) ₂ SO ₄)	0.10	0.10
Reagent E (BSA)	0.10	0.10
Reagent F ($MgCl_2$)	0.25	0.25
Reagent G (KCl)	0.50	0.50
Reagent H (ADH)	0.20	0.20
Reagent J (β -NAD Synth)		0.05
		0.05

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PROCEDURE: (continued)

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

	<u>Test</u>	<u>Blank</u>
Reagent I (EtOH)	0.05	-----
Deionized Water	-----	0.05

Immediately mix by swirling and incubate at 37°C for exactly 2 minutes. Immediately transfer to a boiling water bath for one minute. Remove the Test and Blank and place on ice until cooled. Filter to clarify using a 0.2 μm filter. Record the $A_{340\text{nm}}$ using a suitable spectrophotometer for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(2)(6.22)(0.05)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

2 = Time (in minutes) of assay as per the Unit Definition

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

0.05 = Volume (in milliliter) of enzyme use

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

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

UNIT DEFINITION:

One unit will form 1.0 μmole of β -NAD from nicotinic acid adenine dinucleotide per minute at pH 8.5 at 37°C.

**Enzymatic Assay of β -NAD SYNTHETASE
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 62 mM Tris, 1 mM nicotinic acid adenine dinucleotide, 2 mM adenosine 5'-triphosphate, 1 mM ammonium sulfate, 0.03% (w/v) bovine serum albumin, 20 mM magnesium chloride, 20 mM potassium chloride, 1.6% (v/v) ethanol, 40 units alcohol dehydrogenase, and 0.0075 - 0.05 unit β -NAD synthetase.

REFERENCE:

Yu, C.K. and Dietrich, L.S. (1972) *Journal of Biological Chemistry* **247**, 4794-4802

NOTES:

1. Glutamine can also act as a substrate.
2. The alcohol dehydrogenase used as a coupling enzyme must be suitable for the recycling assay of β -NAD and β -NADH. (It must contain less than 0.005 mole of β -NAD and β -NADH per mole of ADH.)
3. This assay is based on the cited reference.
4. Alcohol Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of ethanol to acetaldehyde per minute at pH 8.8 at 25°C.
5. 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.