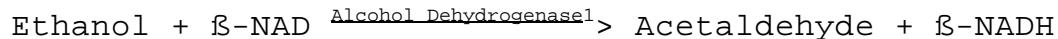
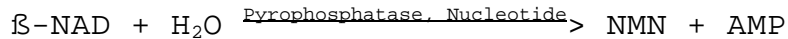


**Enzymatic Assay of PYROPHOSPHATASE, NUCLEOTIDE  
(EC 3.6.1.9)**

**PRINCIPLE:**



Abbreviations:

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

NMN = Nicotinamide Mononucleotide

AMP = Adenosine 5'-Monophosphate

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

**CONDITIONS:** T = 37°C, pH = 7.4, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 4.9 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution ( $\beta$ -NAD)  
(Dissolve the contents of one 20 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Stock No. 260-120, in 5.8 ml of reaction cocktail. Step 1.)
- B. 300 mM Magnesium Chloride Solution (MgCl<sub>2</sub>)  
(Prepare 10 ml in deionized water using Magnesium Chloride Hexahydrate, Prod. No. M-0250.)
- C. 1000 mM Sodium Bicarbonate Solution (NaHCO<sub>3</sub>)  
(Prepare 50 ml in deionized water using Sodium Bicarbonate, Prod. No. S-8875.)
- D. 50 mM Sodium Pyrophosphate Buffer, pH 9.8 at 25°C  
(Prepare 50 ml in deionized water using Sodium Pyrophosphate, Decahydrate, Prod. No. S-9515. Adjust to pH 9.8 at 25°C with 1 M NaOH.)
- E. 95% (v/v) Ethanol (Nondenatured)  
(Prepare 1 ml in deionized water using Nondenatured Ethanol.)

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**REAGENTS:** (continued)

- F. Alcohol Dehydrogenase Solution (ADH)  
(Immediately before use, prepare a solution containing 10 mg/ml in cold deionized water using Alcohol Dehydrogenase, Prod. No. A-7011.)
- G. Pyrophosphatase, Nucleotide Enzyme Solution  
(Immediately before use, prepare a solution containing 0.05 - 0.10 mg/ml in cold deionized water.)

**PROCEDURE:**

Step 1:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into Reagent A ( $\beta$ -NAD):

Deionized Water	3.80
Reagent C ( $\text{NaHCO}_3$ )	1.50
Reagent B ( $\text{MgCl}_2$ )	0.50

Mix by inversion.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.90	2.90

Equilibrate to 37°C. Then add:

Reagent G (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and incubate at 37°C for exactly 30 minutes.

Step 2:

Pipette (in milliliters) the following reagents into suitable cuvettes<sup>2</sup>:

Deionized Water	1.35	1.35
Reagent D (Buffer)	1.50	1.50
Reagent E (EtOH)	0.05	0.05

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**PROCEDURE:** (continued)

Step 2:

	<u>Test</u>	<u>Blank</u>
Reagent F (ADH)	0.01	0.01
<p>Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:</p>		
Test Solution (Step 1)	0.10	-----
Blank Solution (Step 1)		----- 0.10

Mix by inversion and incubate at 25°C for approximately 15 minutes (until there is no increase in A<sub>340nm</sub>). Obtain the final A<sub>340nm</sub> for both the Test and the Blank.

**CALCULATIONS:**

$$\text{Units/mg enzyme} = \frac{r_{A_{340nm} \text{ Blank}} - r_{A_{340nm} \text{ Test}} (3.01)}{(6.22) (30) (\text{mg enzyme/ml RM}) (0.1)}$$

- 6.22 = Millimolar extinction coefficient of β-NADH at 340nm
- 3.01 = Final volume of Step 2
- 30 = Time of assay (in minutes) as per Unit Definition
- 0.1 = volume from Step 1 used in Step 2

**UNIT DEFINITION:**

One unit will hydrolyze 1.0 μmole of β-NAD to NMN and AMP per minute at pH 7.4 at 37°C in the presence of Mg ions.

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 250 mM sodium bicarbonate, 25 mM magnesium chloride, 4.7 mM β-NAD, and 0.005 - 0.010 unit pyrophosphatase, nucleotide.

**Enzymatic Assay of PYROPHOSPHATASE, NUCLEOTIDE  
(EC 3.6.1.9)**

**NOTES:**

1.  $\beta$ -NAD which is not hydrolyzed by pyrophosphatase, nucleotide, is measured in the reaction which alcohol dehydrogenase.
2. This should be done prior to the completion of Step 1.
3. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**