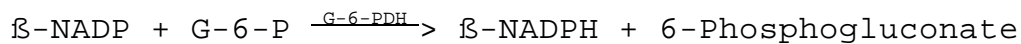
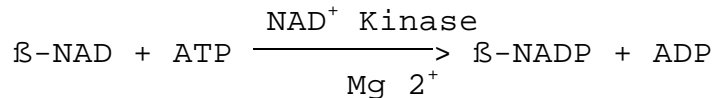


**Enzymatic Assay of NAD⁺ KINASE
(EC 2.7.1.23)**

PRINCIPLE:



Abbreviations used:

ADP = Adenosine 5'-Diphosphate

ATP = Adenosine 5'-Triphosphate

G-6-PDH = Glucose 6-Phosphate Dehydrogenase

β -NAD = β -Nicotinamide Adenine Dinucleotide

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate,
Oxidized Form

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

CONDITIONS: T = 37°C, pH = 7.5, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 1 M Tris Solution
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503.)
- B. 28 mM β -Nicotinamide Adenine Dinucleotide and 33 mM ATP with 20 mM Magnesium Chloride, pH 7.5 at 37°C (Reaction Cocktail)
(Prepare 25 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004, Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394, and Magnesium Chloride Hexahydrate, Sigma Prod. No. M-0250. Adjust the pH to 7.5 at 37°C using Reagent A.)

**Enzymatic Assay of NAD⁺ KINASE
(EC 2.7.1.23)**

REAGENTS: (continued)

- C. 250 mM Glycylglycine Buffer, pH 7.5 at 25°C (Buffer)
(Prepare 50 ml in deionized water using Glycylglycine, Sigma Prod. No. G-1002. Adjust the pH to 7.5 at 25°C with 1 M NaOH.)
- D. 60 mM Glucose-6-Phosphate Solution (G-6-P)
(Prepare 5.0 ml in deionized water using Glucose-6-Phosphate, Disodium Salt, Hydrate, Sigma Prod. No. G-7250.)
- E. 300 mM Magnesium Chloride (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride Hexahydrate, Sigma Prod. No. M-0250.)
- F. 10 mM Tris HCl Buffer, pH 7.5 at 37°C (Enzyme Diluent)
(Prepare 50 ml in deionized water using Reagent A. Adjust to pH 7.5 at 37°C with 1 M HCl.)
- G. Glucose-6-Phosphate Dehydrogenase Solution (G-6-PDH)
(Immediately before use prepare a solution containing 100 units per ml of Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-4134, in cold Reagent C.)
- H. NAD⁺ Kinase Solution (NAD Kinase)
(Immediately before use prepare a solution containing 10 - 30 units per ml in cold Reagent F.)

PROCEDURE:

Step 1:

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

| | <u>Test</u> | <u>Blank</u> |
|-------------------------------|-------------|--------------|
| Reagent B (Reaction Cocktail) | 1.00 | 1.00 |

Equilibrate to 37°C. Then add:

| | | |
|-------------------------------------|-------|-------|
| Reagent H (NAD ⁺ Kinase) | 1.00 | ----- |
| Reagent F (Enzyme Diluent) | ----- | 1.00 |

Immediately mix by swirling and incubate at 37°C for exactly 30 minutes. Immediately transfer to a boiling water bath for 2 minutes. Remove from the boiling water bath and let cool to room temperature. Centrifuge to clarify.

**Enzymatic Assay of NAD⁺ KINASE
(EC 2.7.1.23)**

PROCEDURE: (continued)

Step 2:

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

| | <u>Test</u> | <u>Blank</u> |
|---------------------------------|-------------|--------------|
| Reagent C (Buffer) | 2.50 | 2.50 |
| Reagent D (G-6-P) | 0.10 | 0.10 |
| Reagent E (MgCl ₂) | 0.10 | 0.10 |
| Test Supernatant (from Step 1) | 0.20 | ----- |
| Blank Supernatant (from Step 1) | ----- | 0.20 |

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both the Test and Blank. Then add:

| | <u>Test</u> | <u>Blank</u> |
|------------------------------|-------------|--------------|
| Reagent G (G-6-PDH Solution) | 0.10 | 0.10 |

Immediately mix by inversion and record the increase in A_{340nm} until complete (for approximately 5 minutes). Obtain the final A_{340nm} for both the Test and Blank.

CALCULATIONS:

$$r_{A_{340nm} \text{ Test}} = (\text{Final } A_{340nm} \text{ of Test}) - (\text{Initial } A_{340nm} \text{ of Test})$$

$$r_{A_{340nm} \text{ Blank}} = (\text{Final } A_{340nm} \text{ of Blank}) - (\text{Initial } A_{340nm} \text{ of Blank})$$

$$\text{Units/ml enzyme} = \frac{(r_{A_{340nm} \text{ Test}}) - (r_{A_{340nm} \text{ Blank}})(1000 \text{ nmoles}/\mu\text{mole})(3)(2)}{(6.22)(30)(1)(0.2)}$$

3 = Volume (in milliliters) of reaction in Step 2

2 = Volume (in milliliters) of reaction in Step 1

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

30 = Time (in minutes) of reaction for Step 1

1 = Volume (in milliliter) of enzyme used for Step 1

0.2 = Volume (in milliliter) of Test Supernatant (from Step 1) used in Step 2

**Enzymatic Assay of NAD⁺ KINASE
(EC 2.7.1.23)**

CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will phosphorylate 1.0 nanomole of β -NAD to β -NADP per minute at pH 7.5 at 37°C in the presence of ATP.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 14 mM β -nicotinamide adenine dinucleotide, 17 mM adenosine 5'-triphosphate, 10 mM magnesium chloride, 5 mM Tris, and 10 - 30 units NAD⁺ Kinase.

NOTES:

1. Unit definition for Glucose-6-Phosphate Dehydrogenase: One unit will oxidize 1.0 μ mole of glucose-6-phosphate to 6-phosphogluconate per minute in the presence of β -NADP at pH 7.4 at 25°C.
2. 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.