Enzymatic Assay of NAD\(^+\) KINASE  
(EC 2.7.1.23)

**PRINCIPLE:**

\[
\begin{align*}
\beta-\text{NAD} + \text{ATP} & \xrightarrow{\text{NAD}^+ \text{ Kinase}} \beta-\text{NADP} + \text{ADP} \\
\beta-\text{NADP} + \text{G-6-P} & \xrightarrow{\text{G-6-PDH}} \beta-\text{NADPH} + 6-\text{Phosphogluconate}
\end{align*}
\]

Abbreviations used:
- ADP = Adenosine 5'-Diphosphate
- ATP = Adenosine 5'-Triphosphate
- G-6-PDH = Glucose 6-Phosphate Dehydrogenase
- \(\beta\)-NAD = \(\beta\)-Nicotinamide Adenine Dinucleotide
- \(\beta\)-NADP = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
- \(\beta\)-NADPH = \(\beta\)-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

**CONDITIONS:**  \(T = 37^\circ C\), 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 \(\beta\)-Nicotinamide Adenine Dinucleotide and 33 mM ATP with 20 mM Magnesium Chloride, pH 7.5 at 37\(^\circ\)C  
(Reaction Cocktail)  
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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 (MgCl$_2$)
(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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Reaction Cocktail)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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.
PROCEDURE:  (continued)

Step 2:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>(Buffer)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent D</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>(G-6-P)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent E</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>(MgCl₂)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant (from Step 1)</td>
<td>0.20</td>
<td>------</td>
</tr>
<tr>
<td>Blank</td>
<td>------</td>
<td>0.20</td>
</tr>
<tr>
<td>Supernatant (from Step 1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the A₃₄₀nm until constant, using a suitably thermostatted spectrophotometer. Record the initial A₃₄₀nm for both the Test and Blank. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>(G-6-PDH Solution)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A₃₄₀nm until complete (for approximately 5 minutes). Obtain the final A₃₄₀nm for both the Test and Blank.

CALCULATIONS:

\[
\begin{align*}
\text{r A}_{340\text{nm}} \text{ Test} &= (\text{Final A}_{340\text{nm}} \text{ of Test}) - (\text{Initial A}_{340\text{nm}} \text{ of Test}) \\
\text{r A}_{340\text{nm}} \text{ Blank} &= (\text{Final A}_{340\text{nm}} \text{ of Blank}) - (\text{Initial A}_{340\text{nm}} \text{ of Blank})
\end{align*}
\]

\[
\text{Units/ml enzyme} = \frac{\text{(r A}_{340\text{nm}} \text{ Test})-(\text{r A}_{340\text{nm}} \text{ Blank})(1000 \text{ nmoles/µmole})(3)(2)}{(6.22)(30)(1)(0.2)}
\]

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
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**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 µmole 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.