NAD⁺/NADH Assay Kit

Catalog Number KA1657
100 assays
Version: 05

Intended for research use only
**Table of Contents**

**Introduction** .................................................................................................................. 3

- Intended Use .................................................................................................................. 3
- Background .................................................................................................................. 3
- Principle of the Assay ................................................................................................. 3

**General Information** .................................................................................................. 4

- Materials Supplied ...................................................................................................... 4
- Storage Instruction ....................................................................................................... 4
- Materials Required but Not Supplied .......................................................................... 4
- Precautions for Use ..................................................................................................... 4

**Assay Protocol** .......................................................................................................... 5

- Assay Procedure .......................................................................................................... 5

**Data Analysis** .............................................................................................................. 6

- Calculation of Results ................................................................................................. 6
Introduction

Intended Use

Application:
Direct Assays: NAD⁺/NADH concentrations and ratios in cell or tissue extracts.

Key Features:
- Sensitive and accurate: Detection limit of 0.05 μM and linearity up to 10 μM NAD⁺/NADH in 96-well plate assay.
- Convenient: The procedure involves adding a single working reagent, and reading the optical density at time zero and 15 min at room temperature.
- High-throughput: Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Background

Pyridine nucleotides play an important role in metabolism and, thus, there is continual interest in monitoring their concentration levels. Quantitative determination of NAD⁺/NADH has applications in research pertaining to energy transformation and redox state of cells or tissue. Simple, direct and automation-ready procedures for measuring NAD⁺/NADH concentration are very desirable.

Principle of the Assay

The NAD⁺/NADH Assay Kit is based on a lactate dehydrogenase cycling reaction, in which the formed NADH reduces a formazan (MTT) reagent. The intensity of the reduced product color, measured at 565 nm, is proportional to the NAD⁺/NADH concentration in the sample. This assay is highly specific for NAD⁺/NADH and with minimal interference (<1%) by NADP⁺/NADPH. Our assay is a convenient method to measure NAD, NADH and their ratio.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>10 mL</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>MTT Solution</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>NAD(P)/NAD(P)H Extraction Buffers</td>
<td>each 12 mL</td>
</tr>
<tr>
<td>Enzyme A</td>
<td>120 μL</td>
</tr>
<tr>
<td>Enzyme B</td>
<td>120 μL</td>
</tr>
<tr>
<td>NAD Standard</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

Store all reagents at -20°C.

Materials Required but Not Supplied

✓ Pipetting (multi-channel) devices
✓ Clear-bottom 96-well plates (e.g. Corning Costar)
✓ Plate reader

Precautions for Use

• Precautions
✓ Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

• General Considerations
✓ At these concentrations, the standard curves for NAD and NADH are identical. Since NADH in solution is unstable, we provide only NAD as the standard.
✓ This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.
✓ The following substances interfere and should be avoided in sample preparation. EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).
✓ For samples containing higher than 100 μM pyruvate, we recommend using an internal standard.
Assay Protocol

Assay Procedure

1. Sample Preparation. For tissues weigh ~20 mg tissue for each sample, wash with cold PBS. For cell samples, wash cells with cold PBS and pellet ~$10^5$ cells for each sample. Homogenize samples (either tissue or cells) in a 1.5 mL Eppendorf tube with either 100 μL NAD extraction buffer for NAD determination or 100 μL NADH extraction buffer for NADH determination. Heat extracts at 60°C for 5 min and then add 20 μL Assay Buffer and 100 μL of the opposite extraction buffer to neutralize the extracts. Briefly vortex and spin the samples down at 14,000 rpm for 5 min. Use supernatant for NAD/NADH assays. Determination of both NAD and NADH concentrations requires extractions from two separate samples.

2. Calibration Curve. Prepare 500 μL 10 μM NAD Premix by mixing 5 μL 1 mM Standard and 495 μL distilled water. Dilute standard as follows.

<table>
<thead>
<tr>
<th>No</th>
<th>Premix + H₂O</th>
<th>NAD (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 μL + 0 μL</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>60 μL + 40 μL</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>30 μL + 70 μL</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>0 μL + 100 μL</td>
<td>0</td>
</tr>
</tbody>
</table>

Transfer 40 μL standards into wells of a clear flat-bottom 96-well plate.

3. Samples. Add 40 μL sample per well in separate wells.

4. Reagent Preparation. For each well of reaction, prepare Working Reagent by mixing 60 μL Assay Buffer, 1 μL Enzyme A, 1 μL Enzyme B, 14 μL Lactate and 14 μL MTT. Fresh reconstitution is recommended.

5. Reaction. Add 80 μL Working Reagent per well quickly. Tap plate to mix briefly and thoroughly.

6. Read optical density (OD₀) for time “zero” at 565 nm (520-600nm) and OD₁₅ after a 15-min incubation at room temperature.
Data Analysis

Calculation of Results

First compute the ΔOD for each standard and sample by subtracting OD₀ from OD₁₅. Plot the standard ΔOD’s and determine the slope. The NAD(H) concentration of the sample is computed as follows:

\[
[NAD(H)] = \frac{\Delta OD_{\text{SAMPLE}} - \Delta OD_{\text{BLANK}}}{\text{Slope (\mu M}^{-1})} \times n \quad (\mu M)
\]

where ΔOD\text{SAMPLE} and ΔOD\text{BLANK} are the change in optical density values of the Sample and Blank (STD 4) respectively. Slope is the slope of the standard curve and \(n\) is the dilution factor (if necessary).

Note: If the sample ΔOD values are higher than the ΔOD value for the 10 μM standard, dilute sample in distilled water and repeat this assay. Multiply the results by the dilution factor.

✓ Standard Curve in 96-well plate assay