EnzyChrom™ NAD⁺/NADH Assay Kit (E2ND-100)
Quantitative Colorimetric Determination of NAD⁺/NADH at 565 nm

DESCRIPTION
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. BioAssay Systems’ EnzyChrom™ 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.

APPLICATIONS
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.

KIT CONTENTS

<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 of each sample 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 (OD₁₅) for time “zero” at 565 nm (520-600nm) and OD₆₅₀ after a 15-min incubation at room temperature.

CALCULATION
First compute the ΔOD for each standard and sample by subtracting OD₀ from OD₆₅₀. Plot the standard ΔOD’s and determine the slope. The NAD(H) concentration of the sample is computed as follows:

\[
\text{NAD(H)} = \frac{\text{ΔOD}_{\text{SAMPLE}} - \text{ΔOD}_{\text{BLANK}}}{\text{Slope (µM⁻¹)}} \times n \ (µM)
\]

where ΔOD_{SAMPLE} and ΔOD_{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.

MATERIALS REQUIRED, BUT NOT PROVIDED
Pipetting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Standard Curve in 96-well plate assay

LITERATURE