### Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>NAD/NADH Assay Kit (Colorimetric)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection method</td>
<td>Colorimetric</td>
</tr>
<tr>
<td>Sample type</td>
<td>Tissue Extracts, Cell Lysate</td>
</tr>
<tr>
<td>Assay type</td>
<td>Quantitative</td>
</tr>
<tr>
<td>Assay time</td>
<td>2h 00m</td>
</tr>
</tbody>
</table>

#### Product overview

NAD/NADH Assay Kit (Colorimetric) ab65348 provides a convenient and sensitive tool to quantify NAD⁺ and NADH, and measure their ratio, in samples from mammals and other species.

The NAD cycling enzyme mix in the kit specifically acts on NADH/NAD in an enzyme cycling reaction which significantly increases sensitivity and specificity. There is no requirement to purify NADH/NAD from samples.

The levels of both NADt (total NAD⁺ and NADH) and NADH can be easily measured; the level of NAD⁺ can be easily calculated by subtracting NADH from NADt.

NAD / NADH assay protocol summary:
- extract samples from cells / tissues with extraction buffer and deproteinize with spin column
- for NADH measurement, heat samples to 60°C for 30 min to decompose NAD⁺, cool on ice (this step not necessary for measurement of total NAD⁺/NADH)
- add samples and standards to wells
- add reaction mix and incubate for 5 min at room temp to convert NAD to NADH
- add NADH developer and incubate for 1-4 hrs while reaction cycles
- analyze with microplate reader multiple times during the 1-4 hr incubation
- reaction can be stopped with stop solution

#### Notes

This assay specifically detects NAD and NADH, but not NADP nor NADPH.

If you would like to use a fluorometric reading, please refer to NAD/NADH Assay Kit (Fluorometric) (ab176723).

Review our Metabolism Assay Guide to learn about assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also about how to assay metabolic function in live cells using your plate reader.

#### Platform

Microplate
Storage instructions

Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>Identifier</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAD Cycling Buffer</td>
<td>NM</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>NAD Cycling Enzyme Mix</td>
<td>Green</td>
<td>1 vial</td>
</tr>
<tr>
<td>NADH Developer</td>
<td>Purple</td>
<td>1 vial</td>
</tr>
<tr>
<td>NADH Standard (MW:663.4)</td>
<td>Yellow</td>
<td>1 x 200nmole</td>
</tr>
<tr>
<td>NADH/NAD Extraction Buffer</td>
<td>NM</td>
<td>1 x 50ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>Red</td>
<td>1 x 1.2ml</td>
</tr>
</tbody>
</table>

Relevance

NAD (Nicotinamide adenine dinucleotide) is a coenzyme in metabolic redox reactions, a precursor for several cell signaling molecules, and a substrate for protein posttranslational modifications. NAD is a dinucleotide, consisting of two nucleotides joined through their phosphate groups: with one nucleotide containing an adenosine ring, and the other containing nicotinamide. In metabolism, NAD is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is therefore found in two forms in cells: NAD is an oxidizing agent – it accepts electrons from other molecules and becomes reduced, forming NADH, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD. However, it is also used in other cellular processes, the most notable one being a substrate of enzymes that add or remove chemical groups from proteins in posttranslational modifications.

Images

NAD/NADH was measured in K562 ME2 knockdown cells (pLKO - empty vector; shME2-2 & shME2-3 - two selected knockdown clones). Data are expressed as mean ± SD, n=3. NAD/NADH Ratio is calculated as described in the product protocol.

*Image obtained from Ren JG et al; PLOS one, 2010; 5(9): e12520 (DOI:10.1371/journal.pone.0012520)*
NAD and NADH (tNAD) or NADH alone measured cell lysates. 5e6 cells were lysed in 1 mL, spin filtered, and tested neat or 1/5 (duplicates +/- SD).

Standard curve with background signal subtracted (duplicates; +/- SD).

NADH Standard calibration curve. Quantification of NAD (diamond) and NADH (open square) following product protocol and using NADH standard provided in the kit. No NADP (triangle) was detected in this reaction.

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