Datasheet for ABIN5067573

# NAD+/NADH Assay Kit (Fluorometric)

3 Images

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## Overview

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| Quantity: | 100 tests |
| Application: | Biochemical Assay (BCA) |

## Product Details

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| Purpose: | + +NAD /NADH Assay Kit is a convenient quantitative tool that measures NAD and + NADH within biological samples. |
| Sample Type: | Cell Samples, Tissue Lysate |
| Analytical Method: | Quantitative |
| Detection Method: | Fluorometric |
| Characteristics: | NAD+/NADH Assay Kit is a simple flourometric assay that measures NAD+ and NADH present in biological samples such as cell lysates or tissue extracts in a 96-well microtiter plate format. The kit is specific for NAD+, NADH, and their ratio. The kit will not detect NADP+ or NADPH. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, NAD+ standards and unknown samples. The total NAD+/NADH concentrations of unknown samples are determined by comparison with a known NAD+ standard. Determination of both NAD+ and NADH requires two separate samples for quantification. NAD+ and NADH do not need to be purified from samples, but rather can be extracted individually with a simple acid or base treatment prior to performing the assay. The kit has a detection sensitivity limit of approximately 0.8 nM NAD+. |
| Components: | 1. Fluorometric Probe (40X) : One 150 µL amber tube<br>2. NAD Cycling Substrate : One 200 µL tube<br>3. Assay Buffer : One 25 mL bottle<br>4. Extraction Buffer (10X) : One 10 mL bottle |

## Product Details

5. Electron Mediator (500X) : One 10 µL amber tube

Box 2 (shipped on blue ice packs)

## Target Details

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| Background: | Nicotinamide adenine dinucleotide (NAD) is a complex organic molecule found in all living cells. NAD consists of two nucleotides, adenine and nicotinamide, connected through phosphate groups. + + NAD exists as an oxidized form (NAD ) and a reduced form (NADH). In cells, NAD acts as an electron acceptor, becoming reduced from other molecules to form NADH. The resulting NADH can then act as a reducing agent, donating electrons. An example of this occurs when reduced compounds, + such as glucose and fatty acids, become oxidized and transfer energy via electrons to NAD to form NADH. This electron transfer chemistry is observed in glycolysis and the citric acid cycle. In + eukaryotic cells, NADH electrons made in the cytoplasm are transported to mitochondrial NAD by mitochondrial shuttles such as the malate-aspartate shuttle. The mitochondrial NADH is then oxidized by the electron transport chain that pumps protons across a membrane, generating ATP through a process known as oxidative phosphorylation. + In addition to electron transfer, NAD and NADH are also used as enzyme substrates to add or remove + posttranslational modifications from proteins, as in the process of ADP ribosylation. NAD is also consumed by NAD-dependent deacetylases known as sirtuins. These enzymes can transfer an acetyl group from their substrate protein to the ADP-ribose of NAD, which then cleaves the coenzyme and releases nicotinamide and O-acetyl-ADP-ribose. Non-eukaryotic DNA ligases, which join two DNA + + ends, also operate in an NAD dependent fashion by using NAD as a substrate to donate an adenosine monophosphate (AMP) moiety to one of the 5' phosphate DNA ends. The resulting intermediate is then + attacked by the 3' hydroxyl group of the other DNA end, forming the final phosphodiester bond. NAD + has also been identified as an extracellular signaling molecule. NAD is released from neurons in the large intestine, blood vessels, urinary bladder, neurosecretory cells and from brain synaptosomes. + NAD is therefore a novel neurotransmitter that transmits a signal from nerves to effector cells. |

## Application Details

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| Application Notes: | Optimal working dilution should be determined by the investigator. |
| Comment: | • Detects NAD+, NADH, or total NAD+/NADH<br>• Compatible with cell or tissue lysates<br>• NAD+ standard included |
| Protocol: | The assay is based on an enzymatic cycling reaction in which NAD is reduced to NADH. NADH |

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| | reacts with a fluorometric probe that produces a product which can be measured with a standard 96-well fluorometric plate reader. The intensity of the product fluorescence + is proportional to the NAD and NADH within a sample. A simple acid or base treatment will + differentiate NADPH from NADP within a sample. Samples and standards are incubated for 1-2 hours and then read at λex = 530-570 nm/ λem = 590-600 nm . Samples are compared to a known + concentration of NAD standard within the 96-well microtiter plate format. 2 + . NAD /NADH Cycling Assay Principle. |
| Reagent Preparation: | 1X Extraction Buffer: Dilute the stock 10X Extraction Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. NAD Cycling Reagent: Prepare an NAD Cycling Reagent for the number of assays being tested and just before use. Prepare by diluting the NAD Cycling Substrate 1:25, NAD Cycling Enzyme 1:100, Electron Mediator 1:500, and Fluorometric Probe 1:40 in 1X Assay Buffer. (eg. For 100 assays, combine 200 µL NAD Cycling Substrate, 50 µL NAD Cycling Enzyme, 10 µL Electron Mediator, and 125 µL Fluorometric Probe to 4.615 mL of 1X Assay Buffer for a 5 mL total solution). When preparing, combine the NAD Cycling Substrate, NAD Cycling Enzyme, and Electron Mediator together first and mix prior to adding the Fluorometric Probe. Upon adding the probe, protect the solution from light to prevent the probe from oxidizing. Use the NAD Cycling Reagent immediately. Preparation of Samples These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. It is highly recommended that all samples should be assayed immediately upon preparation or stored for up to 1 month at -80 °C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. Samples may be diluted in deionized water as necessary before testing. Run proper controls and account for any sample dilutions. Always run a standard curve with samples. 4 Tissue homogenates: Sonicate or homogenize 100 mg tissue sample in 0.5 mL cold 1X Extraction Buffer. Centrifuge at 14,000 rpm for 5 minutes at 4 °C to remove insoluble material. Filter the solution with a 10 kDa spin filter to deproteinate the sample. Collect flow through. Perform + dilutions in cold deionized water. Sample may be tested immediately for total NAD /NADH quantification or extracted with acid or base to separate the cofactors. Store unused samples at - 80 °C for up to 1 month. Cell lysates: Culture cells until confluent and harvest. Centrifuge and wash cell pellet with 1X PBS. 6 Centrifuge to pellet cells and remove wash. Resuspend cells at 1-5 x 10 cells/mL in 0.5 mL 1X Extraction Buffer. Homogenize or sonicate the cells on ice. Centrifuge at 14,000 rpm for 5 minutes 4 °C to remove debris. Filter the solution with a 10 kDa spin filter to deproteinate the sample. Collect flow through. Perform dilutions in cold deionized water. Sample may be tested + immediately |

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| | for total NAD /NADH quantification or extracted with acid or base to separate the cofactors. Store unused samples at -80 °C for up to 1 month. Note: Enzymes in tissue and cell sample lysates may deplete NADH rapidly and affect results. Samples should be deproteinized before extracting the cofactors or using within the assay. A spin filter with a 10 kDa cutoff is recommended for efficient and clean separation. NADH Extraction Procedure:<br>• To measure NADH and destroy NAD , add 25 µL of sample to a microcentrifuge tube. Add 5 µ L of 0.1 N NaOH and mix thoroughly. Incubate the tube at 80 °C for 60 minutes and protected from light. Centrifuge the tube to pool all sample solution. Add 20 µL of 1X Assay Buffer to shift the pH of the sample back to neutral. Vortex to mix and centrifuge to pool sample. Keep sample on ice until assaying.<br>• NAD Extraction Procedure:<br>• To measure NAD and destroy NADH, add 25 µL of sample to a microcentrifuge tube. Add 5 µ L of 0.1 N HCl and mix thoroughly. Incubate the tube at 80 °C for 60 minutes and protected from light. Centrifuge the tube to pool all sample solution. Add 20 µL of 1X Assay Buffer to shift the pH of the sample back to neutral. Vortex to mix and centrifuge to pool sample. Keep sample on ice until assaying. Notes: + +If testing both total NAD /NADH and individual cofactors, dilute the total NAD /NADH samples 1:2 with Assay buffer to maintain sample dilution consistency. Avoid samples containing SH groups like DTT, β-mercaptoethanol, or reduced gluathione. Samples should be close to neutral pH before harvesting. Samples with extremely high or low pH values could fail to yield reliable results. |
| Assay Procedure: | 1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate. +2. Add 50 µL of each NAD standard or unknown sample into wells of a 96-well microtiter plate.<br>2. Add 50 µL of NAD Cycling Reagent to each well. Mix the well contents thoroughly and incubate for 1-2 hours at room temperature protected from light. Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.<br>3. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range. +<br>4. Calculate the concentration of NAD /NADH within samples by comparing the sample RFU to the standard curve. |
| Calculation of Results: | 1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected 7 RFU Net RFU RFU background fluorescence. If sample background control value is high, subtract the sample background control value from the sample reading. +<br>2. Plot the corrected fluorescence for the NAD standards against the final concentration of the -1 standards from Table 1 to determine the best slope (µM ). See Figure 2 for an example standard curve.<br>3. + Since all NAD is converted to NADH by the Cycling Reagent, use the standard curve to determine + the total NAD /NADH concentration in pmoles within the sample. Determine the |

## Application Details

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| | total concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected fluorescence values for each sample. Remember to account for dilution factors. + Total NAD /NADH = Sample corrected fluorescence x Sample dilution Slope |
| Restrictions: | For Research Use only |

## Handling

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| Storage: | -20 °C/-80 °C |
| Storage Comment: | Upon receipt, store the NAD Cycling Enzyme and NAD+ Standard at -80°C. Store the Fluorometric Probe and Electron Mediator at -20°C. Store the remaining components at 4°C. |

## Images

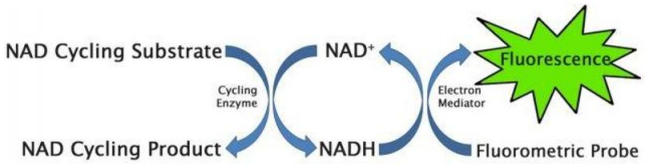
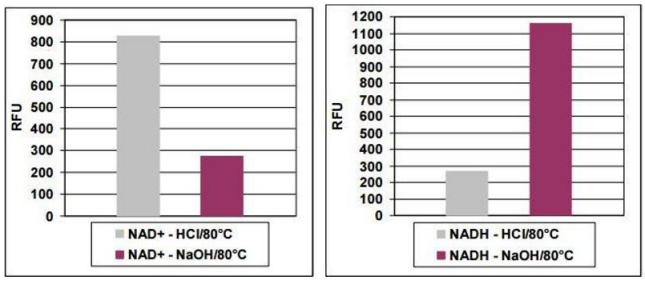
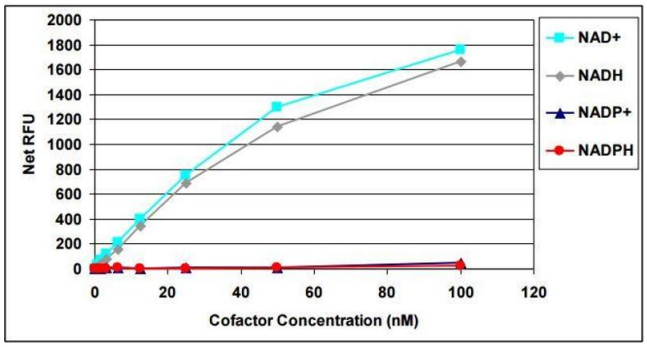



**Image 1.** NAD+ /NADH Cycling Assay Principle




**Biochemical Assay**

**Image 2.** NAD+ /NADH Detection. NAD+ and NADH were both tested at 100 nM with the extraction procedure. NAD+ or NADH were incubated for 60 minutes at 80°C with 0.1N HCl or 0.1N NaOH.




## Biochemical Assay

**Image 3.** NAD+ Standard Curve and Specificity of Assay for NAD+ and NADH. NAD+ , NADH, NADP+ , and NADPH were tested in the NAD+ /NADH Assay Kit.