CREATIVE BIOMART®
Assay Kit

NAD/NADH Assay Kit

Product Information

Cat.No. Kit-2436

Product Overview

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. 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.

Storage

The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Size

100 tests

Kit Components

Assay Buffer: 10 mL
Enzyme A: 120 µL
Lactate: 1.5 mL
Enzyme B: 120 µL
MTT Solution: 1.5 mL
NAD Standard: 0.5 mL
NAD(P)/NAD(P)H Extraction Buffers: each 12 mL

Materials Required but Not Supplied

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

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Plate reader.

Technical Notes

1. 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.
2. 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.
3. 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%).
4. For samples containing higher than 100 µM pyruvate, we recommend using an internal standard.

Assay Protocol

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.
No Premix + H2O NAD (µM)
1 100 µL + 0 µL 10
2 60 µL + 40 µL 6
3 30 µL + 70 µL 3
4 0 µL + 100 µL 0
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

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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.

Analysis

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)] = (ΔOD SAMPLE – ΔOD BLANK)/Slope (µM-1) × n (µM)
where ΔODSAMPLE and ΔODBLANK 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.

Sensitivity

Detection limit of 0.05 µM and linearity up to 10µM NAD+/NADH in 96-well plate assay.

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