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User's Manual and Instructions

NAD+/NADH Assay Kit (Z5030037)

Ultrasensitive 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. Biochain's 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 (100 tests in 96-well plates)

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/NADH Extraction Buffers: each 12 mL
Storage conditions. Store all reagents at -20°C. Shelf life: 6 months after receipt.
Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

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GENERAL CONSIDERATIONS

1. At these concentrations, the standard curves for NAD and NADH are identical. Since NADH in solution is unstable, we provide only NAD athe 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.

PROCEDURES

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 + H_2O	NAD (μM)
1	100 μL + 0 μL	10
2	60 μL + 40 μL	6
3	30 μL + 70 μL	3
8	0μL + 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 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 (OD_0) for time "zero" at 565 nm (520-600nm) and OD_{15} after a 15-min incubation at room temperature.

CALCULATION

First compute the ΔOD for each standard and sample by subtracting OD_0 from OD_{15}. Plot the standard ΔOD's and determine the slope. The NAD(H) concentration of the sample is computed as follows:

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

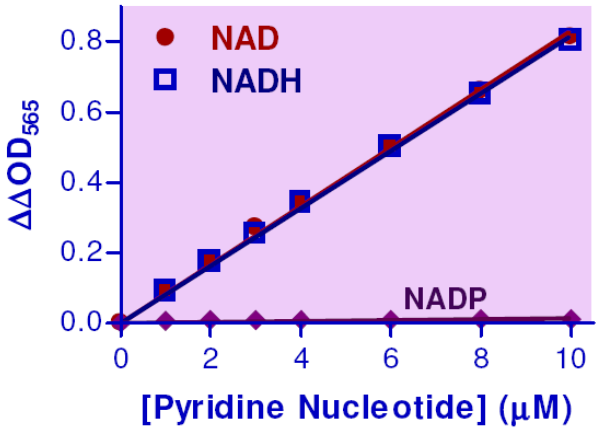
where ΔOD_{SAMPLE} and ΔOD_{BLANK} are the change in optical density values of the Sample and Blank (STD 4) respectively. Slope is the slope of the standard curve and *n* is the dilution factor (if necessary).

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

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3. Vilcheze, C et al. (2005). Altered NADH/NAD_+ Ratio Mediates Coresistance to Isoniazid and Ethionamide in Mycobacteria. Antimicrobial Agents and Chemotherapy. 49(2): 708-720.