EnzyChrom™ NAD+/NADH Assay Kit (E2ND-100)

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. 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 proportionate 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 0.05 µM, 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. No 37°C heater is required.
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 **Lactate: 1.5 mL**
MTT Solution: 1.5 mL **Enzyme A: 120 µL**
NAD Standard: 0.5 mL 1 mM **Enzyme B: 120 µL**
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.

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 Eppindorf 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	Vol (µL)	[NAD] (µM)
1	100µL + 0µL	100	10
2	80µL + 20µL	100	8
3	60µL + 40µL	100	6
4	40µL + 60µL	100	4
5	30µL + 70µL	100	3
6	20µL + 80µL	100	2
7	10µL + 90µL	100	1
8	0µL + 100µL	100	0

Transfer 40 µL standards into wells of a clear flat-bottom 96-well plate.
Samples. Add 40 µL sample per well in separate wells.
3. *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.
4. *Reaction*. Add 80 µL Working Reagent per well quickly. Tap plate to mix briefly and thoroughly.
5. Read optical density (OD_0) for time "zero" at 565 nm (520-600nm) and OD_{15} after a 15-min incubation at room temperature.
6. *Calculation.* Subtract OD_0 from OD_{15} for the standard and sample wells. Use the ΔOD values to determine sample NAD/NADH concentration from the standard curve.

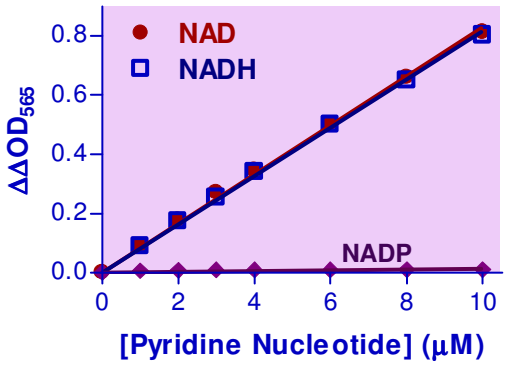
Note: If the sample ΔOD values are higher than the ΔOD value for the 10 µM standard, dilute sample in distilled water and repeat this assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

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 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%).



Standard Curve in 96-well plate assay

LITERATURE

1. Zhao, Z, Hu, X and Ross CW (1987). Comparison of Tissue Preparation Methods for Assay of Nicotinamide Coenzymes. Plant Physiol. 84: 987-988.

2. Matsumura, H. and Miyachi S (1980). Cycling assay for nicotinamide adenine dinucleotides. Methods Enzymol. 69: 465-470.

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.