

EnzyChrom™ NAD⁺/NADH Assay Kit (ECND-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 an alcohol 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 is not interfered 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 0.2 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 **Ethanol (1 vol%):** 1.5 mL
MTT Solution: 1.5 mL **Enzyme Mix:** 120 μL
NAD Standard: 0.5 mL 1 mM
NAD/NADH Extraction Buffers: each 12 mL

Storage conditions. Store all reagents at -20°C. Shelf life of at least 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 ~10⁵ 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 ₂ O	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 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 Mix, 10 μL 1v% Ethanol 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 (OD₀) for time "zero" at 565 nm (520-600nm) and OD₁₅ after a 15-min incubation at room temperature.

6. **Calculation.** Subtract OD₀ from OD₁₅ for the standard and sample wells. Use the ΔOD values to determine sample NAD/NADH concentration from the standard curve.

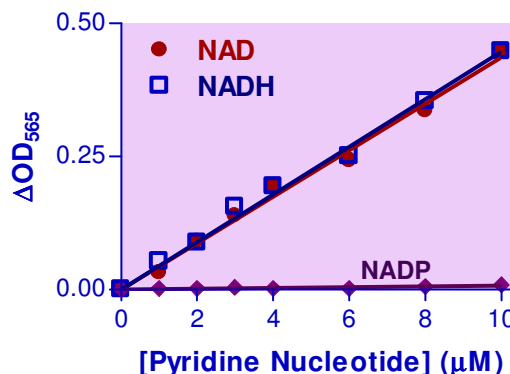
Note: If the sample ΔOD values are higher than the ΔOD value for the 10 μM standard, dilute sample in distilled water and repeat this assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

GENERAL CONSIDERATIONS

- 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.
- 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.
- 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

- Zhao, Z, Hu, X and Ross CW (1987). Comparison of Tissue Preparation Methods for Assay of Nicotinamide Coenzymes. *Plant Physiol.* 84: 987-988.
- Matsumura, H. and Miyachi S (1980). Cycling assay for nicotinamide adenine dinucleotides. *Methods Enzymol.* 69: 465-470.
- 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.