EnzyFluo™ NAD+/NADH Assay Kit (EFND-100)

Quantitative Fluorimetric Determination of NAD+/NADH

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. BioAssay Systems' EnzyFluo™ NAD+/NADH assay kit is based on a lactate dehydrogenase cycling reaction, in which the formed NADH reduces a probe into a highly fluorescent product. The fluorescence intensity of this product, measured at $\lambda_{ex/em}$ = 530/585 nm, is proportional to the NAD+/NADH concentration in the sample. This assay is highly specific for NAD+/NADH with minimal interference (<1%) by NADP+/NADPH and 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 0.02 µM and linearity up to 1 µM NAD+/NADH in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the fluorescence at time zero and 10 min.

High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

KIT CONTENTS

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
Lactate:	1.5 mL	**Enzyme B:**	120 µL
Probe:	750 µL	**NAD Standard:**	0.5 mL

NAD/NADH Extraction Buffers: each 12 mL

Storage conditions. The kit is shipped on ice. 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.

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

PROCEDURES

Note: This kit can also be used directly on cells cultured in 96 well plates. For more information, Please refer to our website or contact us.

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 × g 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 5000 µL 1 µM NAD Premix by mixing 5 µL 1 mM Standard and 4995 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	NAD (□M)
1	100 µL + 0 µL	1.0
2	60 µL + 40 µL	0.6
3	30 µL + 70 µL	0.3
4	0 µL + 100 µL	0

Transfer 50 µL standards into wells of a black flat-bottom 96-well plate.

3. *Samples*. Add 50 µL of each sample in separate wells.
4. *Reagent Preparation*. For each reaction well, prepare Working Reagent by mixing 40 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 10 µL Lactate and 5 µL Probe. Fresh reconstitution is recommended.
5. *Reaction*. Add 50 µL Working Reagent per well quickly. Tap plate to mix.
6. Read fluorescence at $\lambda_{ex/em}$ = 530/585 nm for time "zero" (F_0) and F_{10} after a 10-min incubation at room temperature. Protect plate from light during this incubation.

CALCULATION

First compute the ΔF for each standard and sample by subtracting F_0 from F_{10}. Plot the standard ΔF's and determine the slope. The NAD(H) concentration of the sample is computed as follows:

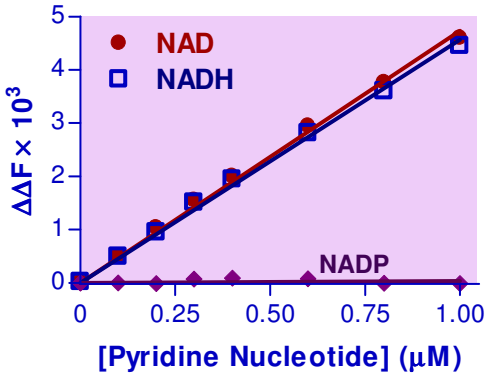
$$[\text{NAD(H)}] = \frac{\Delta F_{SAMPLE} - \Delta F_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

where ΔF_{SAMPLE} and ΔF_{BLANK} are the change in fluorescence intensity 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 ΔF values are higher than the ΔF value for the 1 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. Black, flat bottom 96-well plates and fluorescent plate reader capable of reading at $\lambda_{ex/em}$ = 530/585 nm.



LITERATURE

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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.