

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 $\lambda_{\text{ex/em}} = 530/585 \text{ nm}$, 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

- 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%).
- 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.

- 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 $14,000 \times g$ for 5 min. Use supernatant for NAD/NADH assays. Determination of both NAD and NADH concentrations requires extractions from two separate samples.

- 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 ₂ O | NAD (μM) |
|----|-------------------------------------|-----------------------|
| 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.

- Samples.** Add 50 μL of each sample in separate wells.
- 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.
- Reaction.** Add 50 μL Working Reagent per well quickly. Tap plate to mix.
- Read fluorescence at $\lambda_{\text{ex/em}} = 530/585 \text{ nm}$ 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:

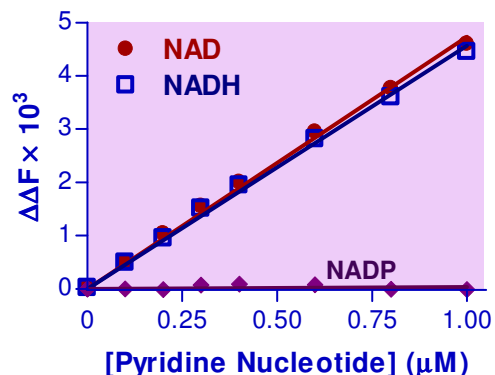
$$[\text{NAD(H)}] = \frac{\Delta F_{\text{SAMPLE}} - \Delta F_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

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 $\lambda_{\text{ex/em}} = 530/585 \text{ nm}$.



PUBLICATIONS

- Joe, Y., et al. (2020). Cross-talk between CD38 and TTP is essential for resolution of inflammation during microbial sepsis. *Cell Reports* 30(4): 1063-1076.e5.
- Zhang, M., et al. (2020). Dysregulated metabolic pathways in age-related macular degeneration. *Scientific Reports* 10(1): 2464.
- Chaurasiya, A., et al. (2021). Pathogen induced subversion of NAD⁺ metabolism mediating host cell death: a target for development of chemotherapeutics. *Cell Death Discovery* 7(1): 10.