EnzyFluo™ NADP+/NADPH Assay Kit (EF2NP-100)

Quantitative Fluorimetric Determination of NADP+/NADPH

DESCRIPTION

Pyridine nucleotides play an important role in metabolism and, thus, there is continual interest in monitoring their concentration levels. Quantitative determination of NADP+/NADPH has applications in research pertaining to energy transformation and redox state of cells or tissue.

Simple, direct and automation-ready procedures for measuring NADP+/NADPH concentration are very desirable. BioAssay Systems' EnzyFluo™ NADP+/NADPH assay kit is based on a glucose dehydrogenase cycling reaction, in which the formed NADPH 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 NADP+/NADPH concentration in the sample. This assay is highly specific for NADP+/NADPH. Our assay is a convenient method to measure NADP+, NADPH and their ratio.

APPLICATIONS

Direct Assays: NADP+/NADPH concentrations and ratios in cell or tissue extracts.

KEY FEATURES

Sensitive and accurate. Detection limit of 0.01 µM and linearity up to 1 µM NADP+/NADPH in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the fluorescence at time zero and 30 min. Room temperature assay.

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
G6P:	120 µL	**Enzyme B:**	120 µL
Probe:	750 µL	**NADP Standard:**	0.5 mL

NADP/NADPH Extraction Buffers: each 12 mL

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 12 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 NADP+ and NADPH are identical. Since NADPH in solution is unstable, we provide only NADP 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%).

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 NADP+extraction buffer for NADP+ determination or 100 µL NADPH extraction buffer for NADPH 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 NADP+/NADPH assays. Determination of both NADP+ and NADPH concentrations requires extractions from two separate samples.
2. *Calibration Curve*. Prepare 5000 µL 1 µM NADP+ Premix by mixing 5 µL 1 mM Standard and 4995 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	NADP (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.

3. *Samples*. Add 50 µL sample per well in separate wells.
4. *Reagent Preparation*. For each reaction well, prepare Working Reagent by mixing 45 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 1 µL G6P 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_{30} after a 30-min incubation at room temperature. Protect plate from light during this incubation.

CALCULATION

First compute the ΔF for each Subtract standard and sample by subtracting F_0 from F_{30}. Plot the standard ΔF's and determine the slope. The NADP+

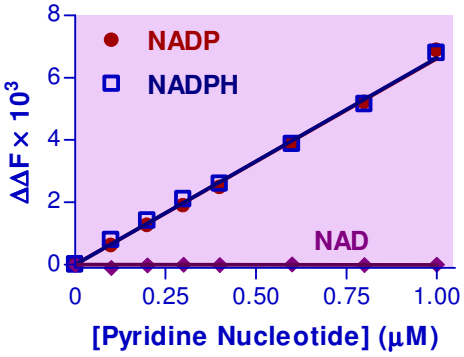
$$[\text{NADP(H)}] = \frac{\Delta F_{\text{SAMPLE}} - \Delta F_{\text{BLANK}}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

(H) concentration of the sample is computed as follows:
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.



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.