BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

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NAD^+/NADH Assay kit (Cat #: A-118)

COMPONENTS: NAD^+/NADH Assay Solution- 5 ml (for 100 wells), store at -70°C **(avoid light exposure)**
NADH Assay Solution- 5 ml (for 100 wells), store at -70°C **(avoid light exposure)**
NADH- 20 mg, store at -70°C **(avoid light exposure)**
NADH Buffer- 1.5 ml, store at 4°C
PEG Solution: 5 ml; store at 4°C **(viscous; pipette solution with a cut tip)**

PRODUCT DESCRIPTION: Nicotinamide adenine dinucleotide exists in two forms in cells: NAD^+ and NADH, which are involved in numerous redox reactions. The NAD^+/NADH Assay Kit is based on LDH-mediated reduction of NAD^+ and the tetrazolium salt INT in a diaphorase-coupled reaction, which generates a formazan product that can be measured by absorbance at 492 nm (detection limit 1 - 5 μM). Reagents are stable for at least 1 year if stored and handled properly.

PROTOCOLS

Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at http://www.bmrservice.com/SupplementPEG.html. Alternatively, the samples can be deproteinized by TCA precipitation (http://www.bmrservice.com/SupplementTCA.html), which is recommended for analysis of nucleotides. Some TCA-treated samples may be diluted with dH_2O prior to assay to achieve optimal result.

Reagent thawing:

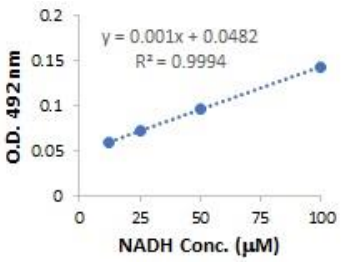
Keep thawed Assay Solutions on ice and shielded from light during assay. Do not over thaw solutions. It is important to minimize the time that the reagents are thawed. Freeze reagents immediately after use.

NADH standards:

Make fresh a 20 mM NADH standard: carefully weigh 1 mg of NADH and vortex to dissolve NADH in 70 μl of ice-cold NADH Buffer in a tube shielded from light. Dilute the standard 200-fold with NADH Buffer to generate 100 μM NADH, e.g., 199 μl NADH Buffer + 1 μl 20 mM NADH. Perform additional 1:1 dilution in NADH Buffer to generate 50 μM, 25 μM, and 12.5 μM NADH. Keep NADH solutions on ice shielded from light during assay.

ASSAY

1. Use a plain (uncoated) 96-well plate for the assay. Add 20 μl of dH_2O to a well. Add 20 μl of each NADH standard to a set of wells. Add each deproteinized sample to two sets of wells (in duplicate).
2. Gently agitate Assay Solutions prior to first pipetting. Add 50 μl NADH Assay Solution to each NADH standard well and to one set of sample wells. Add 50 μl NAD^+/NADH Assay Solution to the other set of sample wells and the dH_2O well. Mix contents by gentle but thorough agitation for 10 sec.
3. Cover and incubate plate in a 37°C humidified incubator for 60 − 90 min. Do not use CO_2 incubator.
4. Measure absorbance at 492 nm using a plate reader. Eliminate air bubbles present in wells prior to measurement. Note: O.D. will be inflated if turbidity appears in wells. Samples can be diluted with dH_2O prior to assay to eliminate turbidity. Some TCA-treated samples may be diluted with dH_2O prior to assay to achieve optimal result.
5. Subtract the dH_2O well reading from all readings of the NAD^+/NADH wells prior to calculation (see below).
6. Plot NADH standards vs. O.D.492 nm. Generate a trendline equation on chart. Use the equation to convert sample O.D. from the two sets of measurements to sample [NADH] and [NADH + NAD^+]. x = concentration in μM; y = O.D.492 nm. A new plot must be generated for each assay.
7. Sample NADH concentration is derived from the NADH Assay Solution wells. Sample (NADH + NAD^+) concentration is derived from the NAD^+/NADH Assay Solution wells. Subtraction of NADH concentration from (NADH + NAD^+) concentration yields sample NAD^+ concentration in μM.



Additional Information:

- Assay Solutions contain the organic solvent DMSO and iodonitrotetrazolium violet. Please contact us or visit the product webpage for MSDS information.