BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: NADH Assay Solution: 10 ml (-20°C; **store at -80°C after reconstitution; avoid light exposure**)
Reagent 1: 1 ml; store at -20°C
Reagent DI: 1.5 mg; store at -20°C
Reagent LA: 100 µl; store at -20°C
NADH: 10 mg, store at -20°C
Reagent LD: 60 µl, store at 4°C (**do not freeze; vortex vial briefly prior to pipetting**)
NADH Buffer: 4 ml, store at 4°C

PRODUCT DESCRIPTION: The Assay Kit measures NAD^+ and NADH present in cells/tissues based on light absorbance at 492 nm. Reagents are stable for at least one year if stored and handled properly.

PROTOCOLS

Preparation of cell/tissue samples: Cell and tissue samples are deproteinized by PEG precipitation (https://bmrservice.com/deproteination-by-peg).

Preparation of NAD^+/NADH Assay Solution:

Follow the pink sheet protocol to reconstitute NADH Assay Solution upon arrival. Keep reconstituted NADH Assay Solution on ice and shielded from light during assay. Freeze solution immediately at -80°C after use.

Use reconstituted NADH Assay Solution to prepare a fresh NAD^+/NADH Assay Solution as needed prior to assay. Each sample requires 50 µl reconstituted NADH Assay Solution and 50 µl freshly prepared NAD^+/NADH Assay Solution.

An example is given for preparation of 1 ml NAD^+/NADH Assay Solution: Briefly vortex Reagent LD vial prior to pipetting. Add 10 µl Reagent LD and 15 µl Reagent LA to 1 ml reconstituted NADH Assay Solution followed by gentle mixing. Scale up or down the preparation as needed. Use the NAD^+/NADH Assay Solution immediately.

Preparation of NADH standards:

Prepare a fresh 20 mM NADH solution by adding 1 mg NADH to 70 µl NADH Buffer. Vortex to dissolve and keep solution on ice and shielded from light. Dilute 20 mM NADH 100-fold with NADH Buffer to generate 200 µM NADH, e.g., 99 µl NADH Buffer + 1 µl 20 mM NADH. Then mix 50 µl 200 µM NADH and 50 µl NADH Buffer to obtain 100 µM NADH. Perform additional 1:1 dilution to obtain 50 µM and 25 µM NADH. Discard NADH solution after each experiment.

ASSAY

1. Use a plain (uncoated) 96-well plate for the assay. Pipette 20 µl of each deproteinized sample to duplicate wells (for sample NADH well and NAD^+/NADH well). Pipette 20 µl of each fresh NADH standard to a set of wells.
2. Add 50 µl reconstituted NADH Assay Solution to one set of sample wells and each NADH standard well.
3. Add 50 µl NAD^+/NADH Assay Solution to the other set of sample wells. Mix contents by gentle agitation.
4. Cover and incubate plate in a 37°C humidified incubator for 60 min in dark. *Do not use CO_2 incubator.*
5. Measure absorbance (O.D.) at 492 nm using a plate reader.
6. Plot NADH standard concentrations (25-200 µM in x axis) vs. NADH standard O.D. (y axis). Generate a trendline equation on chart.
7. Use the equation to convert sample NADH well reading to sample NADH concentration and sample NAD^+/NADH well reading to sample NAD^+/NADH concentration. A new NADH standard plot must be generated for each assay.
8. Subtract sample NADH concentration from sample NAD^+/NADH concentration to generate sample NAD^+ concentration in µM for each sample.

Additional Information:

The assay kit contains the organic solvent dimethyl sulfoxide (DMSO) and iodonitrotetrazolium chloride. Please contact us or visit the product webpage for material SDS information.