CREATIVE BIOMART®
Assay Kit

NAD/NADH Fluorometric Detection Kit

Product Information

Common Name

NAD/NADH

Cat.No.

Kit-0614

Product Overview

This NAD/NADH Fluorometric Detection Kit provides a convenient method for sensitive detection of NAD, NADH and their ratio. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction that significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that considerably reduces the interference from biological samples. There is no need to purify NAD/NADH from sample mix. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 530 - 570/590 - 600 nm (maximum Ex/Em = 540/590 nm) or an absorbance microplate reader at ~576 nm. This kit provides NAD and NADH extraction buffer, and cell lysis buffer for your convenience. It has been frequently used for determining NAD/NADH from cell lysates.

Description

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are two important cofactors found in cells. NADH is the reduced form of NAD+, and NAD+ is the oxidized form of NADH. NAD forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are done by monitoring the changes in NADH or NADPH absorption at 340 nm. This method suffers low sensitivity and high interference since the assay is done in the UV range that requires expensive quartz microplates.

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Storage

Keep in freezer Avoid exposure to light.

Size

250 assays

Kit Components

Component A: NAD/NADH Recycling Enzyme Mixture, 2 bottles (lyophilized powder)
Component B: NADH Sensor Buffer, 1 bottle (20 mL)
Component C: NADH Standard, 1 vial (142 µg)
Component D: NADH Extraction Solution, 1 bottle (10 mL)
Component E: NAD Extraction Solution, 1 bottle (10 mL)
Component F: NAD/NADH Control Solution, 1 bottle (10 mL)
Component G: NAD/NADH Lysis Buffer, 1 bottle (10 mL)

Detection method Fluorescence

Preparation

1. Prepare NADH stock solution:
Add 200 uL of PBS buffer into the vial of NADH standard (Component C) to have 1 mM (1 nmol/uL) NADH stock solution.
Note: The unused NADH stock solution should be divided into single use aliquots and stored at -20 °C.
2. Prepare NAD/NADH reaction mixture:
Add 10 mL of NADH Sensor Buffer (Component B) to the bottle of NAD/NADH Recycling Enzyme Mixture (Component A), and mix well.
Note: This NAD/NADH reaction mixture is enough for two 96-well plates. The unused NAD/NADH reaction mixture should be divided into single use aliquots and stored at -20 °C.
3. Prepare serially diluted NADH standards (0 to 10 uM):
3.1 Add 30 uL of 1 mM NADH stock solution (from Step 1) into 970 uL PBS buffer (pH 7.4) to generate 30 uM (30 pmols/uL) NADH standard solution.
Note: Diluted NADH standard solution is unstable, and should be used within 4 hours.

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3.2 Take 200 uL of 30 uM NADH standard solution (from Step 3.1) to perform 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03 and 0 uM serially diltued NADH standards.
3.3 Add serially diluted NADH standards and/or NAD/NADH containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.
Note: Prepare cells or tissue samples as desired.
Table 1. Layout of NADH standards and test samples in a solid black 96-well microplate
BL BL TS TS TS (NADH) TS (NADH) TS (NAD) TS (NAD)
NS1 NS1 …. …. …. …. …. ….
NS2 NS2
NS3 NS3
NS4 NS4
NS5 NS5
NS6 NS6
NS7 NS7
Note: NS= NAD/NADH Standards; BL=Blank Control; TS=Test Samples; TS (NADH) = Test Samples treated with NADH
Extraction Solution for 10 to 15 minutes, then neutralized by NAD Extraction Solution; TS (NAD) = Test Samples treated with NAD Extraction Solution for 10 to 15 minutes, then neutralized by NADH Extraction Solution.
Table 2 Reagent composition for each well
NADH Standard Blank Control Test Sample Test Sample Test Sample
(NAD/NADH) (NADH Extract) (NAD Extract)
Serial Dilutions*: 25 μL PBS: 25 μL TS: 25 μL TS: 25 μL TS: 25 μL
Component F: 25 μL F: 25 μL F: 25 μL D: 25 μL E: 25 μL
Incubate at room temperature for 10 to 15 minutes
Component F: 25 μL F: 25 μL F: 25 μL E: 25 μL D: 25 μL
Total: 75 μL 75 μL 75 μL 75 μL 75 μL
*Note: Add the serially diluted NADH standards from 0.03 uM to30 uM into wells from NS1 to NS7 in duplicate. High concentration of NADH (e.g., > 300 uM, final concentration) may cause reduced fluorescence signal due to the over oxidation of NADH sensor (to a non-fluorescent product).

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3.4 For NADH Extraction (NADH): Add 25 μL of NADH Extraction Solution (Component D) into the wells of NAD/NADH containing test samples. Incubate at room temperature for 10 to 15 minutes, then add 25 μL of NAD Extraction Solution (Component E) to neutralize the NADH extracts as described in Tables 1 & 2.
For NAD Extraction (NAD): Add 25 μL of NAD Extraction Solution (Component E) into the wells of NAD/NADH containing test samples. Incubate at room temperature for 10 to 15 minutes, then add 25 μL of NADH Extraction Solution (Component D) to neutralize the NAD extracts as described in Tables 1 & 2.
For Total NAD and NADH: Add 25 μL of NAD/NADH Control Solution (Component F) into the wells of NADH standards and NAD/NADH containing test samples. Incubate at room temperature for 10 to 15 minutes, and then add 25 μL of Control Solution (Component F) as described in Tables 1 and 2.
Note1: Prepare cells or tissue samples as desired. NAD/NADH Lysis Buffer (Component G) can be used for lysing the cells.
Note2: In healthy mammalian cells, there is more NAD compare to NADH, so one can simply use total NAD and NADH minus the NAD to calculate the amount of NADH.

Test Sample Preparations Using Component G (NAD/NADH Lysis Buffer):
1. Plant Cell Samples:
Homogenize leaves with the lysis buffer at 200 mg/mL, and centrifuge at 2500 rpm for 5-10 minutes, use the supernatant for tests.
2. Bacterial Cell Samples:
Collect bacterial cells by centrifugation (10,000 g, 0°C, 15 min). Use about 100 to 10 million cells/mL lysis buffer, keep the treated solution at room temperature for 15 minutes. Centrifuge at 2500 rpm for 5 minutes, and use the supernatant for tests.
3. Mammalian Cell Samples:
Remove medium from plate wells, use about 100 uL lysis buffer per 1-5 million cells (or 50-100 µL/well in a 96-well cell culture plate), and keep the treated solution at room temperature for 15 minutes. Use the cell lysate directly or centrifuge it at 1500 rpm for 5 minutes, use the supernatant for tests.
4. Tissue Samples:

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Weigh ~20 mg tissue, wash with cold PBS. Homogenize with 400 ul of lysis buffer in a micro-centrifuge tube. Centrifuge at 2500 rpm for 5-10 minutes, use the supernatant for the assay.

Assay Protocol

4. Run NAD/NADH assay in supernatants reaction:
4.1 Add 75 uL of NADH reaction mixture (from Step 2) into each well of NADH standard, blank control, and test samples (from Step 3.4) to make the total NADH assay volume of 150 uL/well.
4.2 Incubate the reaction at room temperature for 15 minutes to 2 hours, protected from light.
4.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (cutoff 570 nm).
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

Analysis

The fluorescence in blank wells (with the PBS buffer only) is used as a control, and is subtracted from the values for those wells with the NADH reactions.

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