CREATIVE BIOMART®
Assay Kit

NAD Fluorimetric Assay Kit

Product Information

Common Name

NAD

Cat.No. Kit-2590

Product Overview

Fluorimetric NAD Assay Kit provides a sensitive and rapid detection of NAD. The kit directly measure NAD using NAD reagent, our newly developed NAD sensor. The proprietary probe used in this kit reacts only with NAD to generate a product that fluorescence at a specific excitation and emission spectra range, and has little response to NADH. This kit can detect as little as 30 nM NAD in a 100 µL assay volume, and monitor 0.3% NAD generation in the presence of excess amount of NADH. This assay can be performed in a convenient 96-well or 384-well microtiter-plate format and can be used in high-throughput screening.

Kit Components

Component A: NAD Probe 1 bottle (5 mL)
Component B: Assay Solution 1 bottle (5 mL)
Component C: Enhancer Solution 1 bottle (3.5 mL)
Component D: NAD Standard 1 vial (332 µg)

Preparation

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.
1. NAD standard solution (1 mM): Add 500 µL of ddH2O into the vial of NAD standard (Component D) to make 1 mM NAD stock solution.
NAD standard:
Add 10 µL of NAD standard solution into 990 µL H2O or PBS buffer to generate 10 µM NAD standard solution (NS7). Then take the 10 µM NAD standard solution and perform 1:3 serial dilutions in H2O or PBS to get remaining serial dilutions of NAD standard (NS1 - NS6).
Note: Diluted NAD standard solution is unstable, and should be used within 4 hours

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SAMPLE EXPERIMENTAL PROTOCOL:

Table 1. Layout of NAD standards and test samples in a black/solid bottom 96-well microplate. NS = NAD standard (NS1-NS7, 0.01 to 10 μM); BL = blank control; TS = test sample.

BL BL TS TS
NS1 NS1 ... ...
NS2 NS2 ... ...
NS3 NS3
NS4 NS4
NS5 NS5
NS6 NS6
NS7 NS7

Table 2. Reagent composition for each well.

Well Volume Reagent
NS1-NS7 50 μL serial dilution (0.01 to 10 μM)
BL 50 μL Assay Buffer
TS 50 μL sample

Assay Protocol

1. Prepare NAD standards (NS), blank controls (BL), and test samples (TS) according to the layout provided in Table 1 and Table 2. For 384-well plate, use 25 μL of reagent per well instead of 50 μL.
2. Add 20 μL NAD Probe (Component A) solution into each well of NAD standard, blank control, and test samples, mix well. For 384-well plate, use 10 μL of NAD Probe (Component A) solution instead.
3. Add 20 μL Assay Solution (Component B) into each well, mix well. For 384-well plate, use 10 μL of Assay Solution (Component B) instead.
4. Incubate the reaction at room temperature for 10 - 20 minutes, protected from light.
5. Add 15 μL Enhancer (Component C) to each well to make the total NAD assay volume of 105 μL/well, and incubate at room temperature for 10 - 20 minutes, protected from light. For a 384-well plate, add 7.5 uL Enhancer (Component C) instead, for a total volume of 52.5 μL/well.
6. Monitor the fluorescence increase with a fluorescence plate reader at 420/480 nm.

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