Amplite® Fluorimetric NAD Assay Kit *Blue Fluorescence*

Catalog number: 15280
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 15280)
Component A: Quest Fluor™ NAD Probe	Freeze (< -15 °C), Minimize light exposure	1 bottle (5 mL)
Component B: Assay Solution	Freeze (< -15 °C), Minimize light exposure	1 bottle (5 mL)
Component C: Enhancer Solution	Freeze (< -15 °C), Minimize light exposure	1 bottle (3.5 mL)
Component D: NAD Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (332 µg)

OVERVIEW

Nicotinamide adenine dinucleotide (NADH) and its oxidized form (NAD) are essential cofactors for many enzyme reactions found in living cells. Quantifying the generation or consumption of these factors is an important method to monitor the enzyme-mediated reaction or screening the modulator or substrate of these enzyme reactions. There are several kits on the market to quantify NADH or total NAD/NADH amount, but detection NAD generation in the presence of large excess amount of NADH has been quite challenging to date because NAD has its absorption peak at 259 nm and does not fluorescence, making the measurement unpractical. Amplite® Fluorimetric NAD Assay Kit provides a sensitive and rapid detection of NAD. The kit directly measure NAD using Quest Fluor™ 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.

AT A GLANCE

Protocol Summary

1. Prepare NAD standards or test samples (50 µL)
2. Add 20 µL Quest Fluor™ NAD Probe
3. Add 20 µL Assay Solution
4. Incubate at RT for 10 - 20 minutes
5. Add 15 µL Enhancer Solution
6. Incubate at RT for 10 - 20 minutes
7. Monitor Fluorescence at 420/480 nm

Important Thaw each kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	
Emission	480 nm
Excitation	420 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

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

NAD standard solution (1 mM)

Add 500 µL of ddH_2O into the vial of NAD standard (Component D) to make 1 mM NAD stock solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15280

NAD standard

Add 10 µL of NAD standard solution into 990 µL ddH2O or 1X 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 ddH2O 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.

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	ddH2O or 1X PBS
TS	50 µL	sample

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 Quest Fluor™ 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 Quest Fluor™ 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.

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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.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate NAD Dose samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

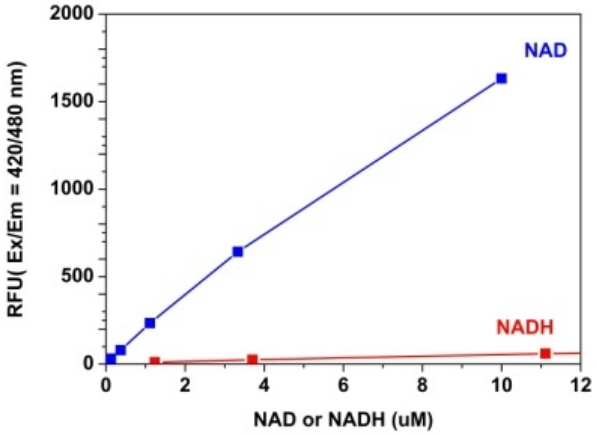

Figure 1. NAD standard curve with 100 µM NADH in presence in the solution. As low as 0.3% of NAD (~300 nM) converted from NADH can be detected with 20 min incubation (n=3). RFU read at Ex/Em = 420/480 nm.

DISCLAIMER

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