Amplite® Fluorimetric Total NAD and NADH Assay Kit *Red Fluorescence*

Catalog number: 15257
Unit size: 400 Tests

Component	Storage	Amount (Cat No. 15257)
Component A: NAD/NADH Recycling Enzyme Mix	Freeze (< -15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B: NADH Sensor Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: NADH Standard (FW: 709)	Freeze (< -15 °C), Minimize light exposure	1 vial (142 µg)

OVERVIEW

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. It 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 then 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 of 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 microplate. This Amplite® NAD/NADH Assay Kit provides a convenient method for sensitive detection of NAD and NADH. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with 570 nm excitation 590 nm emission.

AT A GLANCE

Protocol Summary

1. Prepare NADH standards or test samples (50 µL)
2. Add NAD/NADH working solution (50 µL)
3. Incubate at room temperature for 15 minutes - 2 hours
4. Monitor the fluorescence intensity at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Note

Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 nm
Recommended plate	Solid black

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

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

NADH standard solution (1 mM)

Add 200 µL of 1X PBS buffer into the vial of NADH Standard (Component C) to make 1 mM (1 nmol/µL) NADH standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15257

NADH standard

Add 10 µL of 1 mM (1 nmol/µL) NADH standard solution to 990 µL 1X PBS buffer to generate 10 µM (10 pmol/µL) NADH standard solution (NS7). Take 10 µM NADH standard solution (NS7) to perform 1:3 serial dilutions in 1X PBS buffer to get serially diluted NADH standards (NS6 - NS1). Note: Diluted NADH standard solution is unstable and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 10 mL of NADH Sensor Buffer (Component B) into the bottle of NAD/NADH Recycling Enzyme Mix (Component A) and mix well to make NAD/NADH working solution.

Note: This NAD/NADH working solution is enough for two 96-well plates.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. . Layout of NADH standards and test samples in a solid black bottom 96-well microplate. NS=NADH Standards (NS1 - NS7, 0.014 to 10 µM) , BL=Blank Control, TS=Test Samples.

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. High concentration of NADH (e.g., >100 µM, final concentration) may cause reduced fluorescence signal due to the over oxidation of NADH sensor (to a non-fluorescent product).

Well	Volume	Reagent
NS1 - NS7	50 µL	Serial Dilutions (0.014 to 10 µM)
BL	50 µL	1X PBS buffer
TS	50 µL	test sample

1. Prepare NADH standards (NS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

   Note: Prepare cells or tissue samples as desired.

2. Add 50 µL of NAD/NADH working solution to each well of NADH standard, blank control, and test samples to make the total NAD/NADH assay volume of 100 µL/well. For a 384-well plate, add 25 µL of NAD/NADH working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 15 minutes to 2 hours, protected from light.
4. 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.

   Note: For NAD/NADH ratio measurements, kit 15263 is recommended.

   Note: For cell based NAD/NADH measurements, ReadiUse™ mammalian cell lysis buffer *5X* (cat#20012) is recommended to use for lysing the cells.

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 NADH or NADPH 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

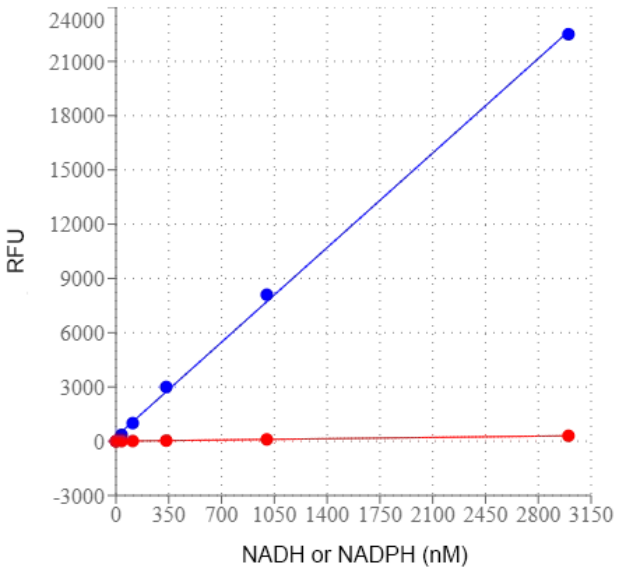

Figure 1. NADH dose response was measured with Amplite® Total NAD and NADH Assay Kit in a solid black 96-well plate using a NOVOStar microplate reader (BMG Labtech). RFU at Ex/Em = 540/590 nm.

DISCLAIMER

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