AAT Bioquest®

Amplite™ Colorimetric NADH Assay Kit

Catalog number: 15271
Unit size: 400 Tests

Component	Storage	Amount
Component A: NADH Probe	Freeze (< -15 °C), Minimize light exposure	1 bottle (4 mL)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (16 mL)
Component C: NADH Standard (FW: 709)	Freeze (< -15 °C), Minimize light exposure	1 vial (142 µg, lyophilized)
Component D: Lysis Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (4 mL)

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+. NAD forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. The traditional NAD/NADH and NADP/NADPH assays are based on monitoring the changes in NADH or NADPH absorption at 340 nm. The short UV wavelength of NAD/NADH and NADP/NADPH assays makes the traditional methods suffer low sensitivity and high interference. Due to the weak absorption of NAD and NADH, the UV absorption method requires large sample sizes, making NAD and NADH measurements unpractical for limited sample size. AAT Bioquest's Amplite™ Colorimetric NADH Assay Kit provides a convenient method for the detection of NADH. The NADH probe is a chromogenic sensor that has its maximum absorbance at 460 nm upon NADH reduction. The absorbance increase at 460 nm is directly proportional to the concentration of NADH in the solution. The NADH probe can recognize NADH in an enzyme-free reaction, and the signal can be easily read by an absorbance microplate reader at ~460 nm. The Amplite™ Colorimetric NADH Assay Kit provides a sensitive assay to detect as little as 3 µM NADH in a 100 µL assay volume. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

AT A GLANCE

Protocol Summary

1. Prepare NADH standards or test samples (50 µL)
2. Add NADH working solution (50 µL)
3. Incubate at RT for 15 minutes to 2 hours
4. Monitor Absorbance at 460 nm

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	460 nm
Recommended plate	Clear bottom

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

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15271

NADH standard
Add 100 µL of 1 mM (1 nmol/µL) NADH standard solution into 400 µL PBS buffer (pH 7.4) to generate 200 µM (200 pmol/µL) NADH standard solution (NS7). Take 200 µM NADH standard solution and perform 1:2 serial dilutions in PBS to get serial dilutions of NADH standard (NS6 - NS1). Note: Diluted NADH standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 1 mL of NADH Probe (Component A) into 4 mL NADH Assay Buffer (Component B) and mix well to make NADH working solution.

Note 5 mL NADH working solution is for one 96-well.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of NADH standards and test samples in a white/clear bottom 96-well microplate. NS= NADH Standards (NS1 - NS7, 3.13 to 200 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.

Well	Volume	Reagent
NS1 - NS7	50 µL	Serial Dilutions (3.13 to 200 µM)
BL	50 µL	PBS
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. Lysis Buffer (Component D) can be used for lysing the cells for convenience.

2. Add 50 µL of NADH working solution to each well of NADH standard, blank control, and test samples to make the total NADH assay volume of 100 µL/well. For a 384-well plate, add 25 µL of 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. Protect from light.

4. Monitor the absorbance increase with an absorbance plate reader at 460 nm.

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EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate NADH 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

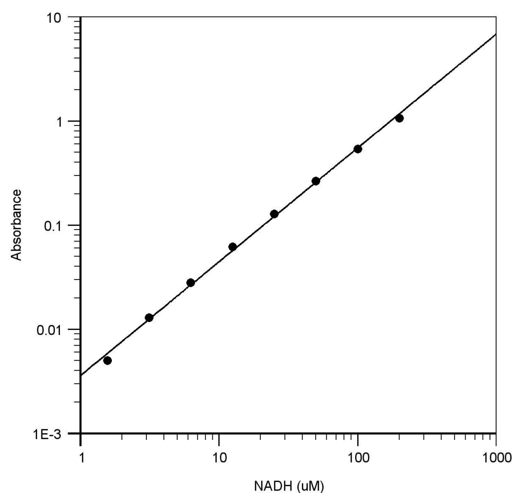

Figure 1. NADH dose response was measured with Amplite™ Colorimetric NADH Assay Kit in a 96-well white/clear bottom plate using a SpectraMax microplate reader (Molecular devices).

DISCLAIMER

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