AAT Bioquest®

Amplite™ Fluorimetric NADH Assay Kit *Red Fluorescence*

Catalog number: 15261
Unit size: 400 Tests

Component	Storage	Amount
Component A: NADH Recycling Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B: NADH Assay Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: NADH Standard	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™ NADH Assay Kit provides a convenient method for sensitive detection of NADH. The enzymes in the system specifically recognize NADH in an enzyme cycling reaction. The enzyme cycling reaction significantly increases detection sensitivity.

AT A GLANCE

Protocol summary

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

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 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.

1. NADH standard solution (1 mM):
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

NADH standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15261

Take the NADH standard solution and use PBS buffer to generate 100 µM NADH standard solution (NS7). Then perform 1:3 serial dilutions to get remaining 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 10 mL of Amplite™ NADH Assay Buffer (Component B) to the bottle of NADH Recycling Enzyme Mixture (Component A) and mix well.

Note This NADH working solution is enough for two 96-well or four 384-well plates. The working solution is not stable, use it promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of NADH standards and test samples in a solid black 96-well microplate. NS = NADH standard (NS1 - NS7, 0.1 to 100 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 (0.1 to 100 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 Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of NADH working solution into 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, protected from light.

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4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal 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. However, the absorption detection will have a lower sensitivity compared to that of the fluorescence reading. For cell based NADH measurements, ReadiUse™ mammalian cell lysis buffer *5X* (Cat No. 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 base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate NADH or NAD 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

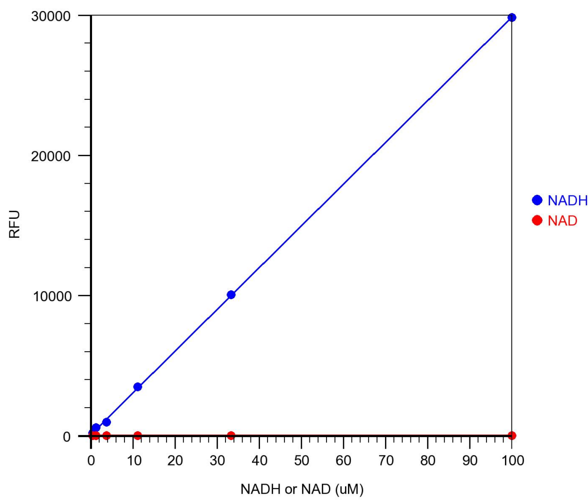

Figure 1. NADH dose response was measured with Amplite™ Fluorimetric NADH Assay Kit in a 96-well solid black plate using a NOVOStar microplate reader (BMG Labtech). RFU measured over Ex/Em = 540/590 nm.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

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