Amplite™ Colorimetric Total NAD and NADH Assay Kit

Catalog number: 15258
Unit size: 400 Tests

Component	Storage	Amount
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.

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 absorbance increase at the absorbance ratio of 570/610 nm.

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	570/610 nm
Recommended plate	Clear bottom

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

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15258

NADH standard

Add 10 µL of 1 mM NADH stock solution into 990 µL PBS buffer (pH 7.4) to generate 10 µM NADH standard solution (NS7). Then perform 1:3 serial dilutions to get serially diltued 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 NAD/NADH Sensor Buffer (Component B) to the bottle of NAD/NADH Recycling Enzyme Mixture (Component A), and mix well.

Note This NAD/NADH working solution is enough for two 96-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, 0.01 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. Note that high concentration of NADH (e.g., >100 µM, final concentration) may cause reduced fluorescence signal due to the over oxidation of NADH sensor.

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

1. Prepare NADH standards (NS), blank controls (BL), and test samples (TS) according to the layout described in Tables 1 and 2. Prepare cells or tissue samples as desired. For a 384-well plate, use 25 µL of reagent per well isntead 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.

3. Incubate the reaction at room temperature for 15 minutes to 2 hours, protected from light.

4. Monitor the absorbance increase with an absorbance plate reader at 575 ± 5 nm or at the absorbance ratio of 570/610 nm to increase assay sensitivity.

   Note For NAD/NADH ratio measurements, Cat No. 15263 is recommended. For cell based NAD/NADH measurements, ReadiUse™ mammalian cell lysis buffer *5X* (Cat No. 20012) is recommended to use for lysing the cells.

EXAMPLE DATA ANALYSIS AND FIGURES

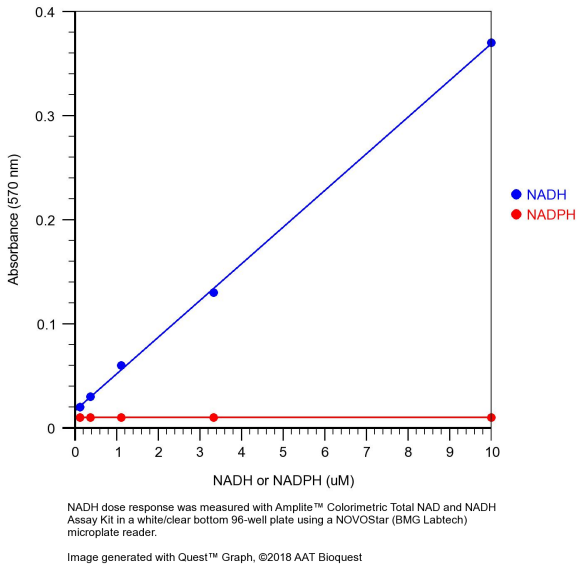

Figure 1. NADH dose response was measured with Amplite™ Colorimetric Total NAD and NADH Assay Kit in a white/clear bottom 96-well plate using a NOVOStar (BMG Labtech) microplate reader.

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