Amplite™ Colorimetric Total NAD and NADH Assay Kit *Enhanced Sensitivity*

Catalog number: 15275
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-I: NADH Probe	Freeze (< -15 °C), Minimize light exposure	1 bottle (4 mL)
Component B-II: NADH Probe Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (16 mL)
Component C: NADH Standard (FW: 709)	Freeze (< -15 °C), Minimize light exposure	1 vial (142 µg)
Component D: Lysis Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 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+, 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. Compared to Kit #15258, this kit has higher 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 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
Add 200 µL of PBS buffer into the vial of NADH standard (Component C) to make 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/15275

NADH standard
Add 10 µL of 1 mM NADH stock solution into 990 µL PBS buffer (pH 7.4) to generate 10 µM (10 pmols/µL) NADH standard solution (NS7). Then take the 10 µM NADH standard solution and perform 1:2 serial dilutions to get remaining serially diluted NADH standards (NS1 - NS6). Note: Diluted NADH standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

1. Add 8 mL of NADH Probe buffer (Component B-II) to the bottle of NAD/NADH Recycling Enzyme Mixture (Component A) and mix well.
2. Add 2 mL of NADH Probe (Component B-I) into above bottle (from Step 1) and mix well.

   Note This NAD/NADH working solution is enough for 200 assays. 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 white wall clear bottom 96-well microplate. NS = NADH standard (NS1 - NS7, 0.156 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:* High concentration of NADH (e.g., >100 µM, final concentration) will cause saturated signal and make the calibration curve non-linear.

Well	Volume	Reagent
NS1 - NS7	50 µL	Serial Dilution (0.156 to 10 µM)
BL	50 µL	PBS
TS	50 µL	Test Sample

1. Prepare NADH standards (NS), blank controls (BL), and test samples (TS) into a white wall clear bottom 96-well microplate 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.

   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 NAD/NADH working solution into each well of NADH standard, blank control, and test samples to make the total

NADH/NADH assay volume of 100 µL/well. For a 384-well plate, add 25 µL of 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 absorbance increase with an absorbance plate reader at 460 nm.

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 Total NAD/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

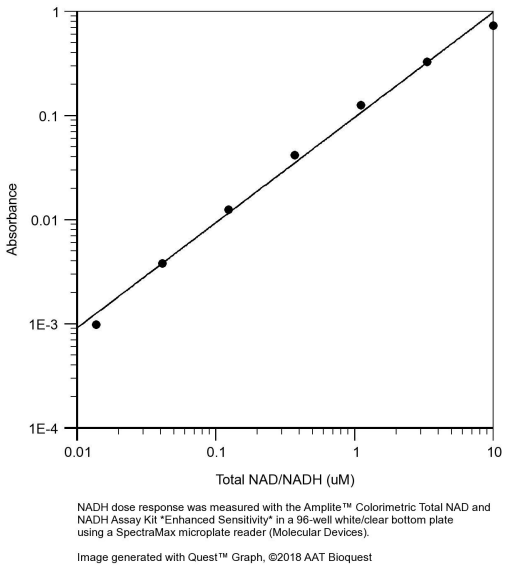

Figure 1. NADH dose response was measured with the Amplite™ Colorimetric Total NAD and NADH Assay Kit *Enhanced Sensitivity* in a 96-well white/clear bottom plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER

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