Amplite® Colorimetric NAD/NADH Ratio Assay Kit

Catalog number: 15273
Unit size: 250 Tests

Component	Storage	Amount (Cat No. 15273)
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: NAD Extraction Solution	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)
Component E: Neutralization Solution	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)
Component F: Extraction Control Solution	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)
Component G: 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. 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. Our Amplite® NAD/NADH Ratio Assay Kit provides a convenient method for sensitive detection of NAD, NADH and their ratio. 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 25 µL of NADH standards and/or test samples
2. Add 25 µL of NAD Extraction Solution
3. Incubate at 37^{o}C for 15 minutes
4. Add 25 µL of Neutralization Solution
5. Add 75 µL of NAD/NADH working solution
6. Incubate at RT for 15 minutes to 2 hours
7. Monitor Absorbance at 460 nm

Important Note

It is highly recommended to incubate the cells with Lysis Buffer (Component G) at 37 °C and use the supernatant for the experiment.

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 (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/15273

NADH standard

Add 50 µL of 1 mM (1 nmol/µL) NADH standard solution into 450 µL 1X PBS buffer (pH 7.4) to generate 100 µM (100 pmols/µL) NADH standard solution. Then take 100 µM NADH standard solution and perform 1:2 serial dilutions in 1X PBS buffer to get serially diluted NADH standards (NS1 - NS7). Note: Diluted NADH standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 8 mL of NADH Probe Buffer (Component B-II) to the bottle of NAD/NADH Recycling Enzyme Mix (Component A), and mix well.

Add 2 mL NADH Probe (Component B-I) into the bottle of Component A+B-II and mix well to make NAD/NADH working solution.

Note This NAD/NADH working solution is enough for 125-200 assays. The working solution is not stable, use it promptly and avoid direct exposure to light.

Note One can try to use ddH2O such as 500 uL to dissolve the Component A, and then mix with B-II and B-I proportionally to make the NAD/NADH working solution for enough use only.

SAMPLE EXPERIMENTAL PROTOCOL

TOTAL NAD+NADH Assay (avail. 400 assays/kit):

Table 1. Layout of NADH standards and test samples in a white/clear bottom 96-well microplate. NS= NADH Standards (NS1 - NS7, 100 to 1.56 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) will cause saturated signal and make the calibration curve non-linear.

Well	Volume	Reagent
NS1 - NS7	50 µL	Serial Dilutions (100 to 1.56 µM)
BL	50 µL	1X 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.

   Note Prepare cells or tissue samples as desired. Lysis Buffer (Component G) can be used for lysing the cells for convenience and incubate the cells with Lysis Buffer at 37^{O}C for 15 minutes and use the supernatant for the experiment.
2. Add 50 µL of NAD/NADH working solution into each well of NADH standard, blank control, and test samples to make the total NAD/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 460 nm.

NAD/NADH RATIO Assay (avail. 250 assays/kit):

Table 3. Layout of NADH standards and test samples in a white/clear 96-well microplate. NS= NADH Standards (NS1 - NS7, 100 to 1.56 µM); BL=Blank Control; TS=Test Samples; TS (NAD) = Test Samples treated with NAD Extraction Solution (Component D) for 10 to 15 minutes, then neutralized by Neutralization Solution (Component E).

BL	BL	TS	TS	TS (NAD)	TS (NAD)
NS1	NS1	...	...		
NS2	NS2	...	...		
NS3	NS3				
NS4	NS4				
NS5	NS5				
NS6	NS6				
NS7	NS7				

Table 4. Reagent compositions for each well. High concentration of NADH (e.g., >100 µM, final concentration) will cause saturated signal and make the calibration curve non-linear.

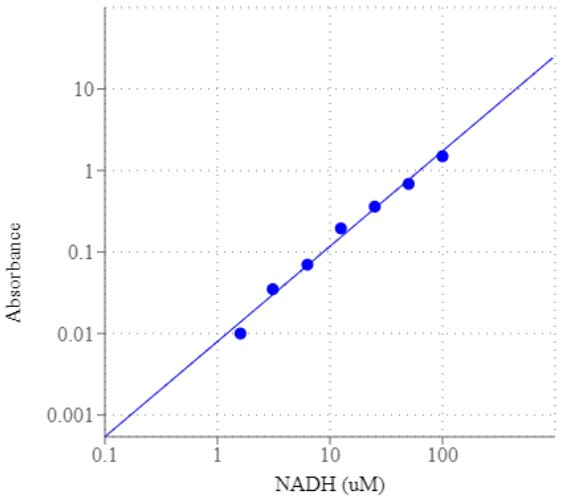
NADH Standard	Blank Control	Test Sample (NAD+NADH)	Test Sample (NAD Extract)
Serial Dilutions: 25 µL	1X PBS: 25 µL	Test Sample: 25 µL	Test Sample: 25 µL
Component F: 25 µL	Component F: 25 µL	Component F: 25 µL	Component D: 25 µL
Incubate at 37 °C for 10 to 15 minutes	Incubate at 37 °C for 10 to 15 minutes	Incubate at 37 °C for 10 to 15 minutes	Incubate at 37 °C for 10 to 15 minutes
Component F: 25 µL	Component F: 25 µL	Component F: 25 µL	Component E: 25 µL
Total: 75 µL	Total: 75 µL	Total: 75 µL	Total: 75 µL

1. Refer to Tables 3 & 4 for compositions of each well.
   1. **For NAD Extraction (NAD amount):** Add 25 µL of NAD Extraction Solution (Component D) into the wells of NAD/NADH containing test samples. Incubate at 37^{O}C for 10 to 15 minutes, then add 25 µL of Neutralization Solution (Component E) to neutralize the NAD extracts as described in Tables 3 & 4.
   2. **For Total NAD and NADH (Total amount):** Add 25 µL of Extraction Control Solution (Component F) into the wells of NADH standards and NAD/NADH containing test samples.

      Incubate at room 37^{O}C for 10 to 15 minutes, and then add 25 µL of Extraction Control Solution (Component F) as described in Tables 3 and 4.

      Note Prepare cells or tissue samples as desired. Lysis Buffer (Component G) can be used for lysing the cells for convenience.
2. Add 75 µL of NAD/NADH working solution into each well of NADH standard, blank control, and test samples (NAD/NADH), and test sample (NAD Extract) to make the total assay volume of 150 µL/well.
3. Incubate the reaction at room temperature for 15 minutes to 2 hours (We tested 60 minutes in the figure shown), protected from light
4. Monitor the absorbance increase with an absorbance plate reader at 460 nm.

EXAMPLE DATA ANALYSIS AND FIGURES



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.