Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit

Catalog number: 15274
Unit size: 250 Tests

Component	Storage	Amount
Component A: NADP/NADPH Recycling Enzyme Mix	Freeze (< -15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B-I: NADPH Probe	Freeze (< -15 °C), Minimize light exposure	1 bottle (4 mL)
Component B-II: NADPH Probe Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (16 mL)
Component C: NADPH Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (167 µg)
Component D: NADP 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™ NADP/NADPH Ratio Assay Kit provides a convenient method for sensitive detection of NADP, NADPH and their ratio. The NADPH 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 NADPH in the solution. The NADPH probe can recognize NADPH in an enzyme-free reaction, and the signal can be easily read by an absorbance microplate reader at ~460 nm. The Amplite™ Colorimetric NADPH Assay Kit provides a sensitive assay to detect as little as 3 µM NADPH 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 NADPH standards and/or test samples
2. Add 25 µL of NADP Extraction Solution
3. Incubate at 37 °C for 15 minutes
4. Add 25 µL of Neutralization Solution
5. Add 75 µL of NADP/NADPH working solution
6. Incubate at RT for 15 minutes to 2 hours
7. Monitor Absorbance at 460 nm

Important

1. It is highly recommended to incubate the cells with Lysis Buffer (Component G) at 37 °C and use the supernatant for the experiment.
2. 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.

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

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15274

NADPH standard
Add 10 µL of 1 mM NADPH stock solution into 490 µL PBS buffer (pH 7.4) to generate 20 µM (20 pmols/µL) NADPH standard solution. Then take the 20 µM NADPH standard solution and perform 1:2 serial dilutions to get remaining serially diluted NADPH standards (NS7 - NS1). Note: Diluted NADPH standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

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

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

SAMPLE EXPERIMENTAL PROTOCOL

TOTAL NADP/NADPH assay (avail. 400 assays/kit):

Table 1. Layout of NADPH standards and test samples in a white/clear bottom 96-well microplate. NS= NADPH Standards (NS1-NS7, 0.156 to 10 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.

Note High concentration of NADPH (e.g., >30 µM, final concentration) will cause saturated signal and make the calibration curve non-linear.

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

1. Prepare NADPH 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.

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

2. Add 50 µL of NADP/NADPH working solution into each well of NADPH standard, blank control, and test samples to make the total NADP/NADPH assay volume of 100 µL/well.

3. Incubate the reaction at room temperature for 15 minutes to 2 hours, protected from light. (We used 1 hour incubation time in data shown)

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

NADP/NADPH RATIO assay (avail. 250 assays/kit):

Table 3. Layout of NADPH standards and test samples in a white/clear 96-well microplate. NS= NADP/NADPH Standards (NS1 - NS7, 0.156 to 10 µM); BL=Blank Control; TS=Test Samples; TS (NADP) = Test Samples treated with NADP Extraction Solution (Component D) for 15 minutes, then neutralized by Neutralization Solution (Component E).

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

Table 4. Reagent compositions for each well.

Note High concentration of NADPH (e.g., >30 µM, final concentration) will cause saturated signal and make the calibration curve non-linear.

NADPH Standard	Blank Control	Test Sample (NADP/NADPH)	Test Sample (NADP Extract)
Serial Dilutions: 25 µL	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 15 minutes	Incubate at 37°C for 15 minutes	Incubate at 37°C for 15 minutes	Incubate at 37°C for 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.

   a. **For NADP Extraction (NADP amount):** Add 25 µL of NADP Extraction Solution (Component D) into the wells of NADP/NADPH containing test samples. Incubate at 37 °C for 10 to 15 minutes, then add 25 µL of Neutralization Solution (Component E) to neutralize the NADP extracts as described in Tables 3 & 4.

   b. **For Total NADP and NADPH (Total amount):** Add 25 µL of NADP/NADPH Control Solution (Component F) into the wells of NADPH standards and NADP/NADPH containing test samples. Incubate at 37o 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 NADP/NADPH working solution into each well of NADPH standard, blank control, and test samples (NADP/NADPH), and test sample (NADP Extract) to make the total assay volume of 150 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

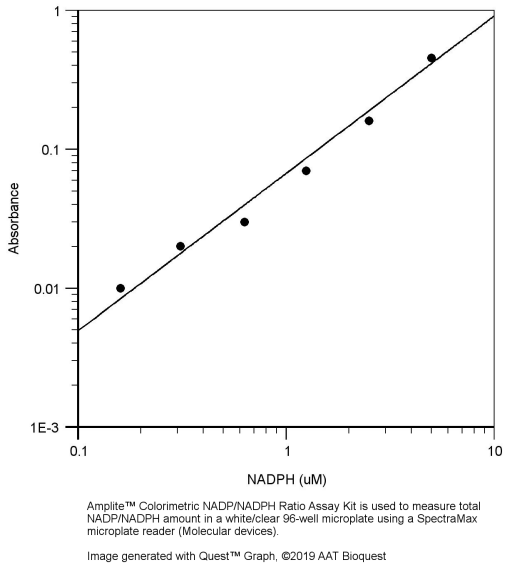

Figure 1. Amplite™ Colorimetric NADP/NADPH Ratio Assay Kit is used to measure total NADP/NADPH amount in a white/clear 96-well microplate using a SpectraMax microplate reader (Molecular devices).

DISCLAIMER

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