Amplite® Colorimetric Total NADP and NADPH Assay Kit

Catalog number: 15260
Unit size: 400 Tests

Component	Storage	Amount (Cat No. 15260)
Component A: NADP/NADPH Recycling Enzyme Mix	Freeze (< -15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B: NADPH Sensor Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: NADPH Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (167 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® NADP/NADPH Assay Kit provides a convenient method for sensitive detection of NADP and NADPH. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction. There is no need to purify NADP/NADPH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity.

AT A GLANCE

Protocol Summary

1. Prepare NADPH standards or test samples (50 µL)
2. Add NADP/NADPH working solution (50 µL)
3. Incubate at room temperature for 15 minutes – 2 hours
4. Monitor the absorbance intensity at 575 ± 5 nm

Important Note

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	575 ± 5 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 1X PBS buffer into the vial of NADPH Standard (Component C) to make 1 mM (1 nmol/µL) NADPH standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15260

NADPH standard

Add 10 µL of 1 mM (1 nmol/µL) NADPH standard solution to 990 µL 1X PBS buffer to generate 10 µM (10 pmol/µL) NADPH standard solution. Take 10 µM NADPH standard solution to perform 1:3 serial dilutions in 1X PBS buffer to get serially diluted NADPH standards (NS7 - NS1). Note: Diluted NADPH standard solution is unstable and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 10 mL of NADPH Sensor Buffer (Component B) into the bottle of NADP/NADPH Recycling Enzyme Mix (Component A) and mix well to make NADP/NADPH working solution.

Note This NADP/NADPH working solution is enough for two 96-well plates.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of NADPH standards and test samples in a white wall clear bottom 96-well microplate. NS= NADPH Standards (NS1 - NS7, 0.003 to 3 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 NADPH (e.g., >100 µM, final concentration) may cause reduced signal due to the over oxidation of NADPH sensor.

Well	Volume	Reagent
NS1 - NS7	50 µL	Serial Dilutions (0.003 to 3 µM)
BL	50 µL	1X PBS buffer
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.

For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

Note Prepare cells or tissue samples as desired.

2. Add 50 µL of NADP/NADPH working solution to each well of NADPH standard, blank control, and test samples to make the total NADPH assay volume of 100 µL/well. For a 384-well plate, add 25 µL of NADP/NADPH 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 575 ± 5 nm or at the absorbance ratio of ~570 nm to ~605 nm to increase assay sensitivity.

Note For NADP/NADPH ratio measurements, kit 15263 is recommended. For cell based NADP/NADPH measurements, ReadiUse™ mammalian cell lysis buffer *5X* (cat #20012) is recommended to use for lysing the cells.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance (570 nm)) 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 NADPH or 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

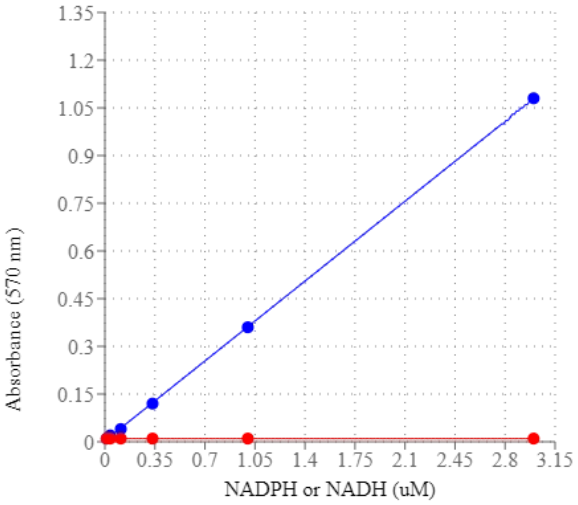

Figure 1. NADPH dose response was measured with Amplite® Colorimetric Total NADP and NADPH Assay Kit in a white/clear bottom 96-well plate using a NOVOStar microplate reader (BMG Labtech).

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