Amplite™ Fluorimetric NADPH Assay Kit *Red Fluorescence*

Catalog number: 15262
Unit size: 400 Tests

Component	Storage	Amount
Component A: NADPH Recycling Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B: NADPH Assay 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™ NADPH Assay Kit provides a convenient method for sensitive detection of NADPH. The enzymes in the system specifically recognize NADPH in an enzyme cycling reaction. The enzyme cycling reaction significantly increases detection sensitivity.

AT A GLANCE

Protocol summary

1. Prepare NADPH working solution (50 µL)
2. Add NADPH standards or test samples (50 µL)
3. Incubate at room temperature for 15 minutes - 2 hours
4. Monitor fluorescence increase at Ex/Em = 540/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Recommended plate:	Solid black

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.

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

PREPARATION OF STANDARD SOLUTION

NADPH standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15262

Use NADPH standard solution and PBS buffer (pH 7.4) to generate 100 µM (100 pmol/µL) NADPH standard solution (NS7). Then use 100 µM NADPH standard solution to perform 1:3 serial dilutions to get remaining serial dilutions of NADPH standard (NS6 - NS1).

Note Diluted NADPH standard solution is unstable, and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 10 mL of Amplite™ NADPH Assay Buffer (Component B) into the bottle of NADPH Recycling Enzyme Mixture (Component A); mix well.

Note This NADPH 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 NADPH standards and test samples in a solid black 96-well microplate. NS = NADPH standard (NS1 - NS7, 0.1 to 100 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

Well	Volume	Reagent
NS1 - NS7	50 µL	Serial Dilution (0.1 to 100 µM)
BL	50 µL	PBS
TS	50 µL	Test Sample

1. Prepare NADPH standards (NS), blank controls (BL), and test samples (TS) into a solid black 96-well microplate according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL. Prepare cells or tissue samples as desired.

2. Add 50 µL of 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, use 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.

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4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm).

Note The contents of the plate can also be transferred to a white clear bottom plate and read by absorbance microplate reader at the wavelength of 576 ± 5 nm. However, the absorption detection will have a lower sensitivity compared to fluorescence reading. For cell based NADPH measurements, ReadiUse™ mammalian cell lysis buffer *5X* (Cat No. 20012) is recommended to use for lysing the cells.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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 NADP 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

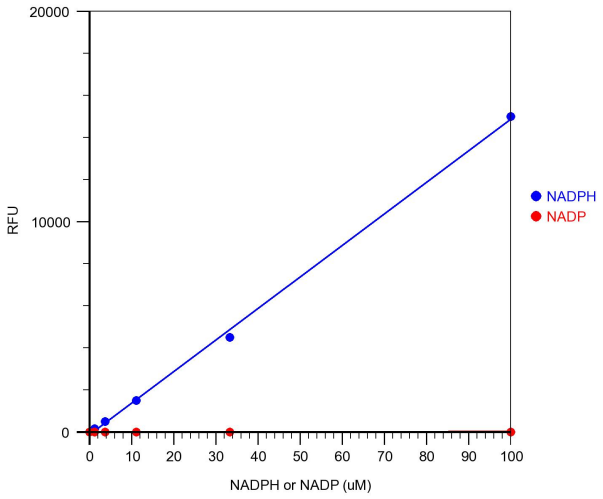

Figure 1. NADPH dose response was measured with Amplite™ Fluorimetric NADPH Assay Kit in a 96-well solid black plate using a NOVOStar microplate reader (BMG Labtech). RFU measured over Ex/Em = 540/590 nm.

DISCLAIMER