Amplite™ Fluorimetric Total NADP and NADPH Assay Kit *Red Fluorescence*

Catalog number: 15259
Unit size: 400 Tests

Component	Storage	Amount
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. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with 570 nm excitation 590 nm emission.

AT A GLANCE

Protocol summary

1. Prepare NADP/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 intensity 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
Cutoff:	570 nm
Recommended plate:	Solid black

Instrument:	Absorbance microplate reader
Absorbance:	576 ± 5 nm
Recommended plate:	Clear bottom

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/15259

Add 10 µL of 1 mM NADPH standard solution to 990 µL PBS buffer to generate 10 µM (10 pmol/µL) NADPH standard solution. Take the 10 µM NADPH standard solution to perform 1:3 serial dilutions to get serial dilutions of NADPH standard (NS7 - NS1).

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

PREPARATION OF WORKING SOLUTION

Add 10 mL of NADP/NADPH Sensor Buffer (Component B) into the bottle of NADP/NADPH Recycling Enzyme Mixture (Component A) and mix well.

Note This NADP/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 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.

Well	Volume	Reagent
NS1 - NS7	50 µL	Serial Dilutions (0.003 to 3 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. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

Note Prepare cells or tissue samples as desired. High concentration of NADPH (e.g., >100 µM, final concentration) may cause reduced fluorescence signal due to the over oxidation of NADPH sensor (to a non-fluorescent product).

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, add 25 µL of 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 fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570, Emission = 590 - 600 nm (optimal at 540/590 nm, cutoff=570 nm).

Note The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

Note For NADP/NADPH ratio measurements, Cat No. 15264 is recommended. For cell based NADP/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 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

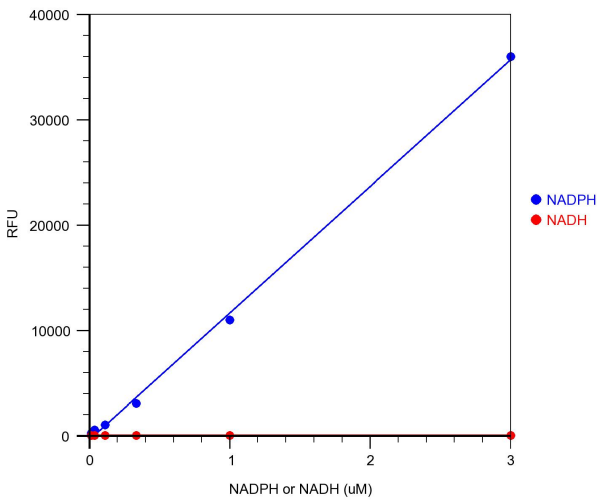

Figure 1. NADPH dose response was measured with Amplite™ Fluorimetric Total NADP and NADPH Assay Kit in a solid black 96-well plate using a NOVOStar microplate reader (BMG Labtech). RFU is Ex/EM = 540/590 nm.

DISCLAIMER