Amplite™ Fluorimetric NADP Assay Kit *Blue Fluorescence*

Catalog number: 15281
Unit size: 200 Tests

Component	Storage	Amount
Component A: Quest Fluor™ NADP Probe	Freeze (<-15 °C), Minimize light exposure	1 bottle (5 mL)
Component B: Assay Solution	Freeze (<-15 °C), Minimize light exposure	1 bottle (5 mL)
Component C: Enhancer Solution	Freeze (<-15 °C), Minimize light exposure	1 bottle (3.5 mL)
Component D: NADP Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (389 µg)

OVERVIEW

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are two important cofactors for many enzyme reactions found in living cells. NAD 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 requires 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 used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. Quantifying the generation or consumption of these factors is an important method to monitor the enzyme-mediated reaction or screening the modulator or substrate of these enzyme reactions. There are several kits on the market to quantify NADPH or total NADP/NADPH amount, but detection NADP generation in the presence of large excess amount of NADPH has been quite challenging to date because NADP has its absorption peak at 260 nm and does not fluorescence, making the measurement unpractical. Amplite™ Fluorimetric NADP Assay Kit provides a sensitive and rapid detection of NADP. The kit directly measure NADP using Quest Fluor™ NADP reagent, our newly developed NADP sensor. The proprietary probe used in this kit reacts only with NADP to generate a product that fluorescence at a specific excitation and emission spectra range and has little response to NADPH. This kit can detect as little as 30 nM NADP in a 100 µL assay volume, and monitor 0.3% NADP generation in the presence of excess amount of NADPH. This assay can be performed in a convenient 96-well or 384-well microtiter-plate format and can be used in high-throughput screening.

AT A GLANCE

Protocol summary

1. Prepare NADP standards or test samples (50 µL)
2. Add 20 µL Quest Fluor™ NADP Probe
3. Add 20 µL Assay Solution
4. Incubate at RT for 10 - 20 minutes
5. Add 15 µL Enhancer Solution
6. Incubate at RT for 10 - 20 min
7. Monitor Fluorescence at 420/480 nm (Cutoff = 455 nm)

Important Thaw each kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	420 nm
Emission:	480 nm
Cutoff:	455 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. NADP standard solution (1 mM):
Add 500 µL of ddH_2O into the vial of NADP Standard (Component D) to make 1 mM NADP standard solution.

PREPARATION OF STANDARD SOLUTION

NADP standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15281

Add 10 µL of 1mM NADP standard solution into 990 µL H_2O or 1X PBS buffer to generate 10 µM NADP standard solution (NS7). Take 10 µM NADP standard solution (NS7) and perform 1:3 serial dilutions in H_2O or 1X PBS buffer to get serially diluted NADP standard (NS6 - NS1).

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

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of NADP standards and test samples in a black/solid bottom 96-well microplate. NS=NADP standards (NS1-NS7, 0.01 to 10 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 (0.01 to 10 µM)
BL	50 µL	1X PBS buffer
TS	50 µL	sample

1. Prepare NADP standards (NS), blank controls (BL), and test samples (TS) 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.

2. Add 20 µL Quest Fluor™ NADP Probe (Component A) solution into each well of NADP standard, blank control, and test samples, mix well. For a 384-well plate, use 10 µL of Quest Fluor™ NADP Probe (Component A) solution instead.

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3. Add 20 µL Assay Solution (Component B) into each well, mix well. For a 384-well plate, use 10 µL of Assay Solution (Component B) instead.

4. Incubate the reaction at room temperature for 10 - 20 minutes, protected from light.

5. Add 15 µL Enhancer (Component C) to each well to make the total NADP assay volume of 105 µL/well. For a 384-well plate, add 7.5 uL Enhancer (Component C) instead, for a total volume of 52.5 µL/well.

6. Incubate at room temperature for 10 - 20 minutes, protected from light.

7. Monitor the fluorescence increase with a fluorescence plate reader at 420/480 nm (Cutoff = 455nm).

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 NADP Dose 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

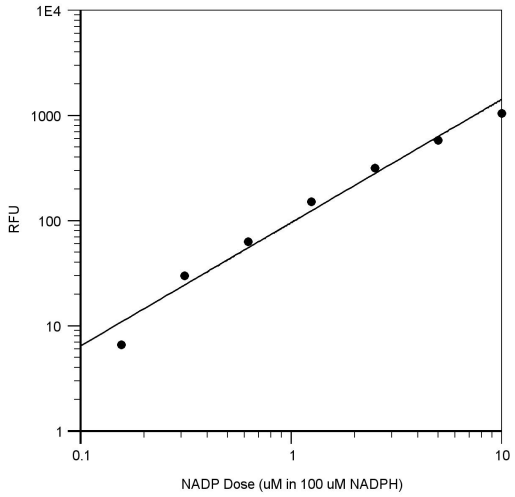

Figure 1. NADP standard curve with 100 µM NADPH in presence in the solution. As low as 0.3% of NADP (~300 nM) converted from NADPH can be detected with 20 min incubation (n=3). RFU read at Ex/Em = 420/480 nm.

DISCLAIMER