Amplite™ Fluorimetric NADP Assay Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 15281 (200 assays), 15281B (2,000 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

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 Ex/Em = 420/480 nm, 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.

Kit Components

Components	**Amount**	
	15281	**15281B**
Component A: Quest Fluor™ NADP Probe	1 bottle (5 mL)	1 bottle (50 mL)
Component B: Assay Solution	1 bottle (5 mL)	1 bottle (50 mL)
Component C: Enhancer Solution	1 bottle (3.5 mL)	1 bottle (35 mL)
Component D: NADP Standard	1vial (389 µg)	1vial (389 µg)

Assay Protocol for One 96-Well Plate

Brief Summary
Prepare NADP standards or test samples (50 µL) → Add 20 µL Quest Fluor™ NADP Probe → Add 20 µL Assay Solution → Incubate at RT for 10-20 minutes → Add 15 µL Enhancer Solution → Incubate at RT for 10-20 min → Monitor Fluorescence at 420/480 nm

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

1. Prepare NADP stock solution:

Add 500 µL of ddH_2O into the vial of NADP standard (Component D) to make 1 mM NADP stock solution.
Note: The unused NADP stock solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare serial dilutions of NADP standard (0 to 10 µM):

2.1 Add 10µL of NADP stock solution (from Step 1) into 990 µL H_2O or PBS buffer to generate 10 µM NADP standard solution.
Note: Diluted NADP standard solution is unstable, and should be used within 4 hours.

2.2 Take 200 µL of 10 µM NADP standard solution to perform 1:3 serial dilutions in H_2O or PBS to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0 µM serial dilutions of NADP standard.

2.3 Add 50 µL serial dilutions of NADP standard and NADP containing test samples into a black/solid bottom 96-well microplate as described in Table 1:

Table 1 Layout of NADP standards and test samples in a black/solid bottom 96-well microplate

BL	BL	TS	TS	….	….						
NS1	NS1	….	….	….	….						
NS2	NS2										
NS3	NS3										
NS4	NS4										
NS5	NS5										
NS6	NS6										
NS7	NS7										

Note1: NS= NADP Standards, BL=Blank Control, TS=Test Samples.
Note2: Add the serially diluted NADP standards from 10 µM to 0.01 µM into wells from NS1 to NS7 in duplicate.

3. Run NADP assay:

3.1 Add 20 µL Quest Fluor™ NADP Probe (Component A) solution into each well of NADP standard, blank control, and test samples, mix well.

3.2 Add 20 µL Assay Solution (Component B) into each well, mix well.
Note: For a 384-well plate, add 25 µL of sample and 10 µL of Quest Fluor™ NADP Probe and 10 µL Assay Solution into each well.

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

3.4 Add 15 µL Enhancer (Component C) to each well to make the total NADP assay volume of 105 µL/well, and incubate at room temperature for 10-20 minutes, protected from light.
Note: For a 384-well plate, add 7.5 uL Enhancer.

3.5 Monitor the fluorescence increase with a fluorescence plate reader at 420/480 nm.

Data Analysis

The fluorescence in blank wells (with H_2O or PBS buffer only) is used as a control, and is subtracted from the values for those wells with the NADP reactions. A NADP standard curve is shown in Figure 1

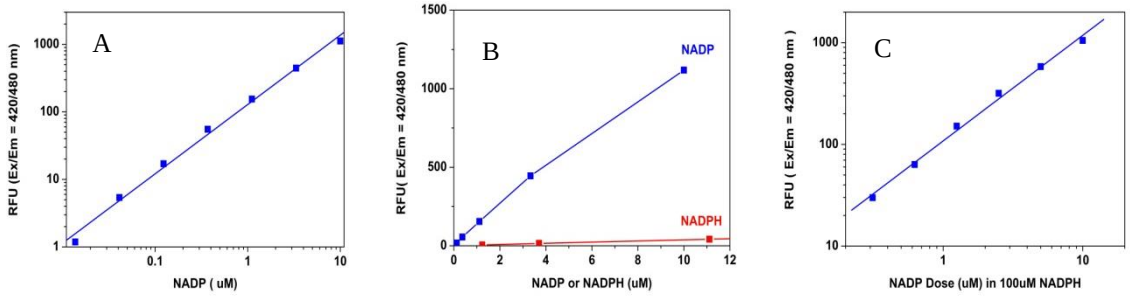

Figure1. NADP dose response was measured with Amplite™ Fluorimetric NADP Assay Kit in a 96-well black/solid bottom plate using a Germini microplate reader (Molecular devices). A: NADP standard curve, as low as 30 nM of NADP can be detected with 20 min incubation (n=3). B: Comparison of NADP and NADPH response C: 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).

References

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2. Stephen Y. Xue, Valeria Y. Hebert, Danicia M. Hayes, Corie N. Robinson, Mitzi Glover, and Tammy R. Dugas. Toxicol. Sci., Aug 2013; 134: 323 - 334.
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