Amplite™ Fluorimetric NADP/NADPH Assay Kit

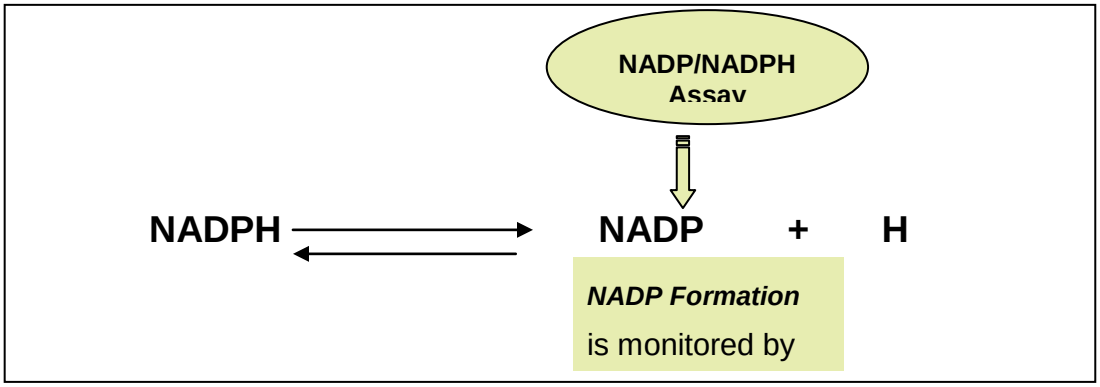
Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 15259 (400 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 found in cells. NADH is the reduced form of NAD+, and NAD+ is the oxidized form of NADH. 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 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 used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis.

The existing NADP/NADPH assays are run in UV range by absorption. The assays suffer low sensitivity and high interference. This Amplite™ Fluorimetric 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 that 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 resulted from biological samples. There is no need to purify NADP/NADPH from sample mix.



The Amplite™ Fluorimetric NADP/NADPH Assay Kit provides a sensitive, one-step assay to detect as little as 1 picomoles of NADP(H) in a 100 µL assay volume (10 nM; Figure 1). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm. The longer red emission minimizes the interference from the autofluorescence of biological samples.

Kit Key Features

Broad Application:	Can be used for quantifying NADP/NADPH in solutions and in cell extracts.
Sensitive:	Detect as low as 1 picomoles of NADP/NADPH in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Kit Components

Components	Amount
Component A: NADP/NADPH Recycling Enzyme mixture	2 bottles (lyophilized powder)
Component B: NADPH Sensor Buffer	1 bottle (20 mL)
Component C: NADPH Standard (FW: 833.36)	1 vial (167 µg)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare NADP/NADPH reaction mixture (50 µL) → Add NADPH standards or test samples (50 µL) → Incubate at room temperature for 15 minutes – 2 hours → Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

1. Prepare NADPH stock solution:

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

Note: The unused NADPH stock solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare NADP/NADPH reaction mixture:

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 reaction mixture is enough for two 96-well plates. The unused NADP/NADPH reaction mixture should be divided into single use aliquots and stored at -20 °C.

3. Prepare serial dilutions of NADPH standard (0 to 10 µM):

3.1 Add 10 µL of NADPH stock solution (from Step 1) to 990 µL PBS buffer to generate 10 µM (10 pmol/µL) NADPH standard solution.

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

3.2 Take 200 µL of 10 µM NADPH standard solution to perform 1:3 serial dilutions to get 3, 1, 0.3, 0.1, 0.03, 0.01, 0.03 and 0 µM serial dilutions of NADPH standard.

3.3 Add serial dilutions of NADPH standard and NADP/NADPH containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Note: Prepare cells or tissue samples as desired.

Table 1. Layout of NADPH standards and test samples in a solid black 96-well microplate

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

Note: NS= NADPH Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well

NADPH Standard	Blank Control	Test Sample
Serial Dilutions*: 50 µL	PBS: 50 µL	50 µL

**Note: Add the serially diluted NADPH standards from 0.003 µM to3 µM into wells from NS1 to NS7 in duplicate. 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).*

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

4. Run NADP/NADPH assay in supernatants:

4.1 Add 50 µL of NADPH reaction mixture (from Step 2) into each well of NADPH standard, blank control, and test samples (see Step 3.3) to make the total NADPH assay volume of 100 µL/well
Note: For a 384-well plate, add 25 µL of sample and 25 µL of NADPH reaction mixture into each well.

4.2 Incubate the reaction at room temperature for 15 minutes to 2 hours, protected from light.

4.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 530-570 /590-600 nm (optimal at 540/590 nm).
Note1: 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.
Note2: To detect NADPH only, aliquot 200 µL of samples into Eppendorf tubes. Heat samples to 60 °C for 30 minutes in a heating block or a water bath. All NADP will be deactivated while NADPH will be still intact under these conditions. Cool samples on ice, and quickly spin samples if precipitates occur. Transfer 50 µL of NADPH samples into the wells as indicated in Tables 1 and 2.

Data Analysis

The fluorescence in blank wells (with the PBS buffer only) is used as a control, and is subtracted from the values for the wells of NADPH reactions. A NADPH standard curve is shown in Figure 1.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

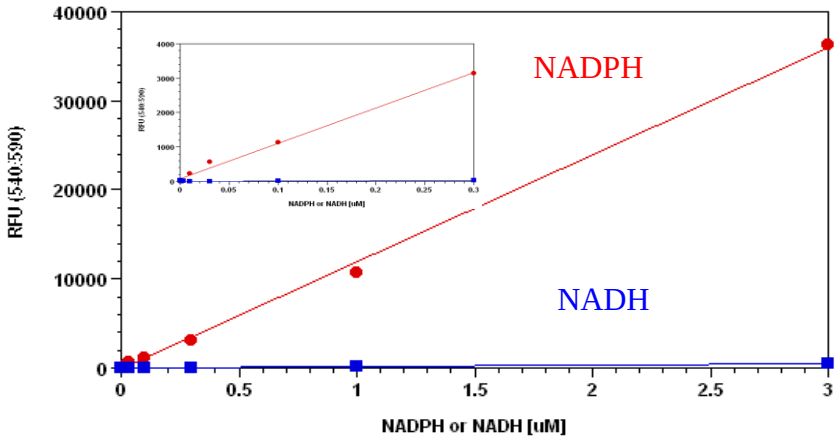


Figure 1. NADPH dose response was measured with Amplite™ Fluorimetric NADP/NADPH Assay Kit in a black 96-well plate using a NOVOStar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of NADPH can be detected with 30 minutes incubation (n=3) while there is no response from NADH.

References

1. Hedeskov CJ, Capito K, Thams P. (1987) Cytosolic ratios of free [NADPH]/[NADP+] and [NADH]/[NAD+] in mouse pancreatic islets, and nutrient-induced insulin secretion. Biochem J, 241, 161.
2. Gaetani GF, Ferraris AM, Sanna P, Kirkman HN. (2005) A novel NADPH:(bound) NADP+ reductase and NADH:(bound) NADP+ transhydrogenase function in bovine liver catalase. Biochem J, 385, 763.
3. Kobayashi K, Miura S, Miki M, Ichikawa Y, Tagawa S. (1995) Interaction of NADPH-adrenodoxin reductase with NADP+ as studied by pulse radiolysis. Biochemistry, 34, 12932.
4. Marino D, Gonzalez EM, Frendo P, Puppo A, Arrese-Igor C. (2006) NADPH recycling systems in oxidative stressed pea nodules: a key role for the NADP(+)-dependent isocitrate dehydrogenase. Planta.