# Amplite™ Colorimetric NADP/NADPH Assay Kit

**Blue Color**

***Ordering Information:***
Product Number: #15260 (400 assays)

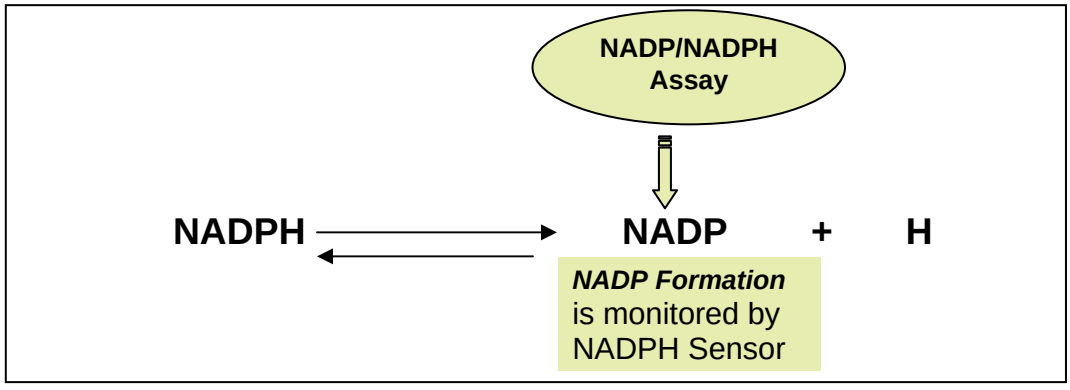
***Instrument Platform:***
Absorbance microplate readers

***Storage Conditions:***
Keep in freezer and avoid light

## 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. 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 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, NADPH and their ratio. 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 absorbance at 576±5 nm.




The Amplite™ Fluorimetric NADP/NADPH Assay Kit provides a sensitive, one-step colorimetric assay to detect as little as 3 picomoles of NADP (H) in a 100 µL assay volume (30 nM; Figure 1). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read absorbance microplate reader at 575±5 nm or with absorbance ratio at 570±10 nm to 605±10 nm to increase assay sensitivity.

## Kit Key Features

***Broad Application:*** Can be used for quantifying NADP/NADPH in solutions, in cell extracts.
***Sensitive:*** The kit detect as low as 3 picomoles of NADP/NADPH in solution.
***Continuous:*** Easily adapted to automation with no separation required.
***Convenient:*** Formulated to have minimal hands-on time. No wash is required.
***Non-Radioactive:*** No special requirements for waste treatment.

## Kit Components

| Component | 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 min-2hr → Read absorbance at 575±5 nm**

*Note: Thaw1 vial (or bottle) each of all the kit components to room temperature before starting the experiment.*

**1. Prepare NADPH stock solution:**

Prepare NADPH standard stock solution: Add 200 µL of PBS buffer into the NADPH standard vial (Component C) to have 1 mM (1 nmol/µL) stock solution.
*Note: The unused NADPH solution should be divided as single use aliquots and stored at -20°C.*

**2. Prepare NADP/NADPH reaction mixture:**

Prepare the NADP/NADPH reaction mixture: Add 10 mL of NADP/NADPH sensor buffer (Component B) to the bottle of NADP/NADPH recycling enzyme mixture (Component A), mixed well.
*Note: This solution is enough for two 96-well plates. The unused NADP/NADPH mixture should be divided as single use aliquots and stored at -20°C.*

**3. Prepare Serial NADPH (0 to 10 µM) solutions**

3.1 Add 10 µL of NADPH standard stock solution (from step 1) to 990 µL PBS buffer to generate 10 µM (10 pmol/µL) standard.
*Note: Diluted NADPH standard solution is unstable, should be used within 4 hours.*

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

3.3 Add NADPH standards and NADP/NADPH-containing test samples into a 96-well white/clear bottom microplate as described in Tables 1 and 2
*Note: Prepare your cell or tissue samples as desired.*

**Table 1**. Layout of NADPH standards and test samples in a white/clear bottom 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 0.01 µM to10 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 overoxidation of NADPH sensor (to a non-fluorescent product).*

**4. Run NADP/NADPH Assay in supernatants**

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

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

4.3 Monitor the absorbance increase with 575 $\pm$ 5 nm by using an absorbance plate reader.
*Note1: To detect NADPH only, aliquot 200 µL samples into Eppendorf tubes. Heat samples to 60$^{o}$C for 30 min in a heating block or a water bath. All NADP will be destroyed 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 Table 1 and 2.*

**5. Run Data Analysis**

The absorbance 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. The typical data are shown in Figure 1 (NADPH standard curve).
*Note: The absorbance background increases with time, thus it is important to subtract the absorbance of the blank wells for each data point.*

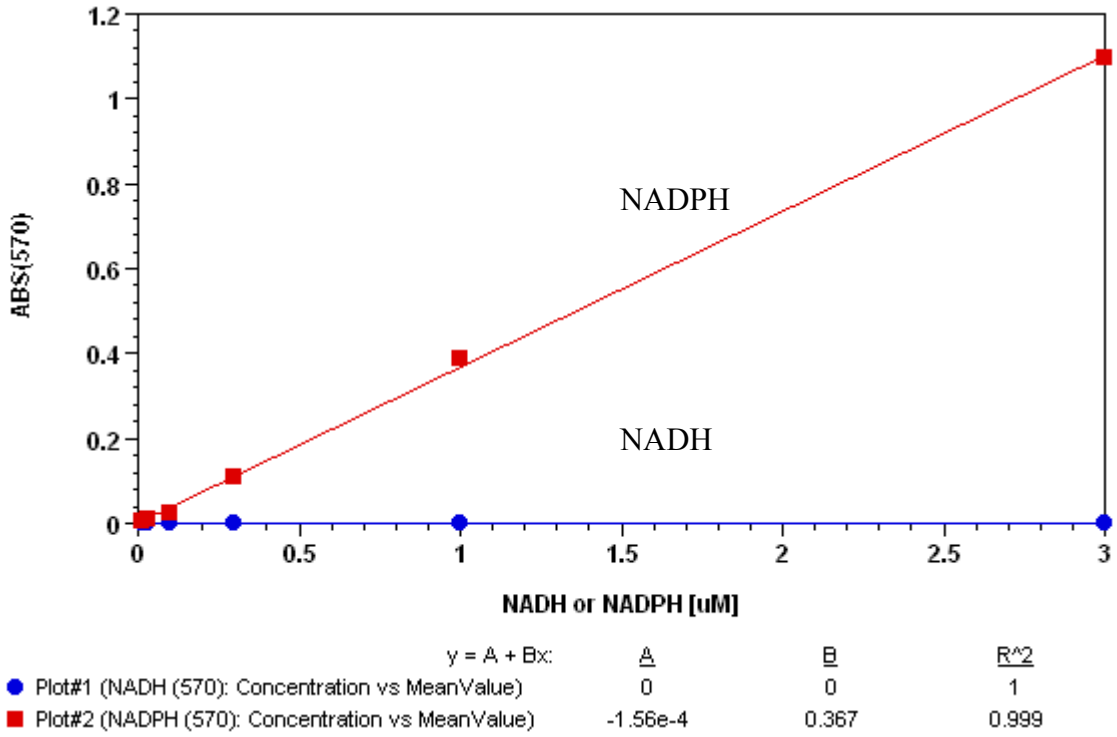



Figure 1. NADPH dose response on 96-well black plate was measured with Amplite™ NADP/NADPH Assay Kit using a BMG LabTech NOVOStar microplate reader. As low as 30 nM (3 pmol/well) of NADPH can be detected with 1hour incubation time (n=3) while there is no response from NADH.

## Related Products

| | | |
|---|---|---|
| 15257 | Amplite™ Fluorimetric NAD/NADH Assay Kit *Red Fluorescence* | 1 kit |
| 15258 | Amplite™ Colorimetric NAD/NADH Assay Kit | 1 kit |
| 15259 | Amplite™ Fluorimetric NADP/NADPH Assay Kit *Red Fluorescence* | 1 kit |
| 15260 | Amplite™ Colorimetric NADP/NADPH Assay Kit | 1 kit |
| 15261 | Amplite™ Fluorimetric NADH Assay Kit *Red Fluorescence* | 1 kit |
| 15262 | Amplite™ Fluorimetric NADPH Assay Kit *Red Fluorescence* | 1 kit |

## 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.