Amplite™ Fluorimetric Total NADP and NADPH Assay Kit

*Red Fluorescence*

**Ordering Information**
- **Product Number:** 15259 (400 assays)
- **Storage Conditions:** Keep in freezer. Avoid exposure to light
- **Instrument Platform:** 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 NADP 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 Total NADP and 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 Total NADP and NADPH Assay Kit provides a sensitive, one-step assay to detect as little as 1 picomole 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.
**Kit Components**

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A: NADP/NADPH Recycling Enzyme mixture</td>
<td>2 bottles (lyophilized powder)</td>
</tr>
<tr>
<td>Component B: NADPH Sensor Buffer</td>
<td>1 bottle (20 mL)</td>
</tr>
<tr>
<td>Component C: NADPH Standard (FW: 833.36)</td>
<td>1 vial (167 μg)</td>
</tr>
</tbody>
</table>

**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, 0.003 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

<table>
<thead>
<tr>
<th>BL</th>
<th>BS1</th>
<th>TS</th>
<th>TS</th>
<th>....</th>
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</thead>
<tbody>
<tr>
<td>NS1</td>
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<td>NS2</td>
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<tr>
<td>NS7</td>
<td>NS7</td>
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</tbody>
</table>

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

**Table 2.** Reagent composition for each well

<table>
<thead>
<tr>
<th>NADPH Standard</th>
<th>Blank Control</th>
<th>Test Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Dilutions*: 50 μL</td>
<td>PBS: 50 μL</td>
<td>50 μL</td>
</tr>
</tbody>
</table>

*Note: Add the serially diluted NADPH standards from 0.003 μM to 3 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).*
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: For NADP/NADPH ratio measurements, kit 15264 is recommended.
   Note3: For cell based NADP/NADPH measurements, ReadiUse™ mammalian cell lysis buffer *5X* (cat#20012) is recommended to use for lysing the cells.

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.

![NADPH and NADH](image)

Figure 1. NADPH dose response was measured with Amplite™ Fluorimetric total NADP and 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


Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.