**Product Overview**

**Product name**
NADP/NADPH Assay Kit (Fluorometric) ab176724

**Detection method**
Colorimetric/Fluorometric

**Sample type**
Cell culture extracts, Tissue Extracts

**Assay type**
Quantitative

**Assay time**
1h 00m

**Species reactivity**
Reacts with: Mammals, Other species

**Product overview**
NADP/NADPH Assay Kit (Fluorometric) ab176724 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 recycling reaction that significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that considerably reduces the sample interference.

The NADP/NADPH assay can be performed in a 96-well or 384-well microtiter-plate format. The signal can be read by either a fluorescence microplate reader at Ex/Em = 530-570/590-600 nm (maximum Ex/Em = 540/590 nm) or an absorbance microplate reader at ~576 nm.

NADP/ NADPH assay protocol summary:
- add samples and standards to wells
- add extraction solutions and incubate for 15 min
- add reaction mix and incubate for 30 min - 2 hr
- analyze with microplate reader

**Notes**
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.

**Platform**
Microplate reader
Storage instructions

Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADP Extraction Solution</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>NADP/NADPH Control Solution</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>NADP/NADPH Lysis Buffer</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>NADP/NADPH Recycling Enzyme Mixture</td>
<td>2 vials</td>
</tr>
<tr>
<td>NADPH Extraction Solution</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>NADPH Sensor Buffer</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>NADPH Standard</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

Relevance

NADP (Nicotinamide adenine dinucleotide phosphate) is a coenzyme composed of ribosylnicotinamide 5-phosphate (NMN) coupled by pyrophosphate linkage to the 5-phosphate adenosine 2,5-biphosphate. It serves as an electron carrier in a number of reactions, being alternately oxidised (NADP⁺) and reduced (NADPH). The oxidative phase of the pentose phosphate pathway is the major source of NADPH in cells, producing approximately 60% of the NADPH required. NADPH provides the reducing equivalents for biosynthetic reactions and the oxidation-reduction involved in protecting against the toxicity of ROS, allowing the regeneration of GSH. NADPH is also used for anabolic pathways, such as lipid synthesis, cholesterol synthesis and fatty acid chain elongation.

Images

Total intracellular NADPH levels in unstressed 661W photoreceptor-like cells were not significantly altered by exposure to 25mM glucose. Phototoxic stimuli and oxidative stress induced by H₂O₂ but not exposure to the apoptosis inducing agent reduced total intracellular NADPH levels significantly, however exposure to excess glucose significantly increased total NADPH levels in all injury models relative to similarly treated photoreceptor-like cells exposed to 5mM glucose.
Quantity of NADP, NADPH and total (NADP+NADPH) in U937 cells (duplicates; +/- SD). 0.5 x 10^7 cells were lysed in 1 mL of lysis buffer.

Quantity of NADP, NADPH and total (NADP+NADPH) in RAW 264.7 cells (duplicates; +/- SD). 0.5 x 10^7 cells were lysed in 1 mL lysis buffer.

Standard curves with background signal subtracted (duplicates; +/- SD).

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