Cell Technology, Inc

Fluoro NADP/NADPH

Fluorescent NADP/NADPH Detection Kit

Key Benefits

- Detection of NADP/NADPH content in cells or tissue extracts.
- Study of NADP/NADPH levels antioxidation and oxidative stress.
- Detection NADP/NADPH in cell death, energy metabolism, mitochondria function.
- Species Independent - NADP/NADPH detection in Bacterial, fungal and plant cells
- Highly Sensitive – Detects up to 4nM NADP and NADPH
- Highly Specific - No Cross reactivity with NAD/NADH
- Easy to Use – 96 well Fluorescent Plate reader readout

Assay Principle

Nicotinamide adenine dinucleotide phosphate (NADP+) is used in anabolic reactions, such as lipid and nucleic acid synthesis, which require NADPH as a reducing agent. NADPH is the reduced form of NADP+, and NADP+ is the oxidized form of NADPH. In cells, NADPH plays the role of a carrier of reducing power and is primarily involved in maintaining optimal redox metabolism. A simplified assay for the measurement of NAD and NADP is critical to understanding the roles of these pyridine nucleotides in normal and abnormal cells.

NADPH is produced in the oxidative phase of the pentose phosphate pathway in cells, a multifunctional pathway whose primary purpose is to generate reducing power in the form of NADPH. NADPH is a cofactor for enzymes that synthesize energy-rich molecules and provide the reducing equivalents for the oxidation-reduction involved in protecting the cell from the toxicity of reactive oxygen species (ROS) and NADPH oxidase-dependent ROS generation. Both NAD and NADP have been shown to influence hemoglobin affinity for oxygen in erythrocytes. In plant cells, NADPH is used as the reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis [1-2].

Cell Technology's Fluoro NADP/NADPH provides a highly reliable, sensitive fluorometric assay for the quantification of NADP, NADPH and their ratio in biological samples.

Reaction:

The Fluoro NADP/NADPH detection kit utilizes a non-fluorescent detection reagent, which is oxidized in the presence NADPH to produce its fluorescent analog and NADP. NADP is further converted to NADPH via an enzyme-coupled reaction. The enzyme reaction specifically reacts with NADP/NADPH and not with NAD/NADH.

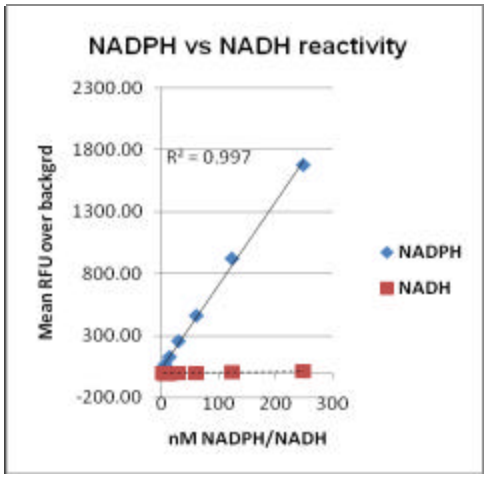
Reaction:

1. NADPH + non-fluorescent detection reagent + electron coupler → fluorescent analog + NADP

2. NADP + enzyme coupled reaction → NADPH (then proceeds to reaction 1).

Excitation: 530-570nm and Emission at 590-600nm

A



B

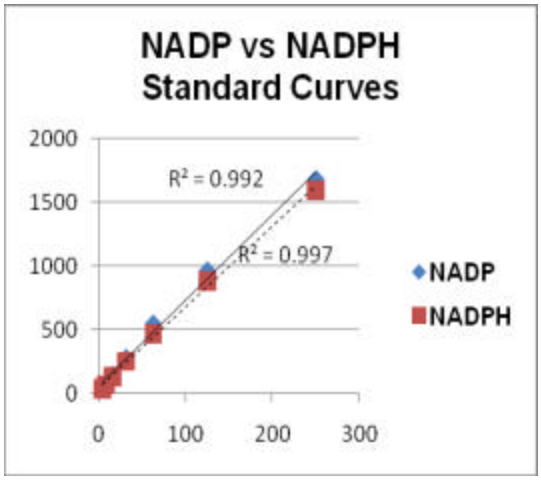


Figure 1. Comparasion of NADPH vs NADH Standard Curves (graph A) and NADP vs NADPH Standard curves (graph B).

Ordering Information

Catalog #	Size	Price (US$)
NADPH 100-2	100	495

References:

1. Comprehensive Invited Review WEIHAI YING. NAD /NADH and NADP /NADPH in Cellular Functions and Cell Death: Regulation and Biological Consequences. ANTIOXIDANTS &REDOX SIGNALING Volume 10,Number 2,2008.

2. Bedard K and Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245 –313,2007.

3. Lowry, Oliver H., Passonneau, Janet V. and Rock, Martha K. The Stability of Pyridine Nucleotides. The Journal of Biological Chemistry, 236, #10, 1961.

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