CREATIVE BIOMART®
Assay Kit

NADP/NADPH Fluorometric Detection Kit

Product Information

Cat.No.

Kit-0617

Product Overview

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

Description

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

Applications

1. Detection of NADP/NADPH activity in cells or tissue extracts.2. Study of NADP/NADPH levels antioxidation and oxidative stress.3. Detection NADP/NADPH in cell death, energy metabolism, mitochondria function. 4. NADP/NADPH detection in Bacterial, fungal and plant cells.

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Usage

1. For Research use only. Not for use in diagnostic procedures.2. Practice safe laboratory procedures by wearing gloves, protective clothing and eyewear.3. The reaction is not stable in the presence of thiols (DTT or 2-mercaptoethanol). Keep these reactants below 10µM. 4. Once the vial of Part 4019 NADPH Detection reagent is opened, it is important that low lighting conditions be used while aliquoting as well as performing the experiment. Direct and prolonged light exposure may increase the background, resulting in compromised linearity.

Storage

1. Long Term Storage: Store contents as labeled.2. Upon Arrival: Various-Please see kit components above for specific storage conditions.3. Upon arrival store the following components at -20°C. 4. The rest of the components should be stored at -80°C.

Kit Components

Reagent-Storage Temperature1. Enzyme Mix, 170μL: 2-8°C;2. NADP/NADPH Lysis Solution: 12.5mL, 2-8°C;3. NADP Extraction Buffer: 22 mL, (Green label), 2-8°C;4. NADPH Extraction Buffer: 22 mL, (Magenta label), 2-8°C;5. Reaction Buffer: 11 mL, 2-8°C;6. Standard curve diluent: 15 ml, 2-8°C;7. NADPH Detection reagent: 110 μL, -20°C;8. 3X Substrate Mix, 6mL: -20°C; 9. NADPH Standard: 3 vials dried, -20°C

Features & Benefits

1. Detection of NADP/NADPH content in cells or tissue extracts.2. Detection of NADP/NADPH levels in antioxidation and oxidative stress.3. Detection of NADP/NADPH levels in cell death, energy metabolism, and mitochondria function.4. Species Independent-NADP/NADPH detection in Bacterial, fungal and plant cells.5. Highly Sensitive-Detects up to 4nM NADP and NADPH.6. Highly Specific-No Cross reactivity with NAD/NADH.7. Easy to Use-96 well Fluorescent Plate reader readout.

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