

ReadiUse™ NADPH Regenerating Kit

Catalog number: 15265

Unit size: 1000 Tests

Component	Storage	Amount
Component A: Assay Buffer I	Freeze (<math>< -15\text{ }^\circ\text{C}</math>), Minimize light exposure	1 bottle (25 mL)
Component B: Assay Buffer II	Freeze (<math>< -15\text{ }^\circ\text{C}</math>), Minimize light exposure	1 bottle (25 mL)
Component C: 500X Glucose-6-phosphate dehydrogenase (400 units/mL)	Freeze (<math>< -15\text{ }^\circ\text{C}</math>), Minimize light exposure	1 vial (100 μL)

OVERVIEW

NADPH provides the reducing equivalents for biosynthetic reactions and for oxidation-reduction involved in protection against the toxicity of ROS (reactive oxygen species). NADPH is also used for anabolic pathways, such as lipid synthesis, cholesterol synthesis and fatty acid chain elongation. It is the source of reducing equivalents for cytochrome 450 hydroxylation of aromatic compounds, steroids, alcohols, and drugs. NADPH is a necessary cofactor in many xenobiotic metabolism reactions. In chloroplasts, NADP is reduced by ferredoxin-NADP reductase in last step of the electron chain of the light reactions of photosynthesis. The NADPH produced is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. Many oxidoreductases and all ligases use NADPH as coenzymes. NADPH is required for the measurement of oxidase activity catalyzed by P450s, FMOs, NADPH-P450 reductase, and many other oxidase enzymes. AAT Bioquest's ReadiUse™ NADPH Regenerating Kit provides two ready-to-use solutions to regenerate NADPH by a simple mixing. This kit can be used for all NADPH-requiring oxidase assays (cDNA-expressed enzymes and liver fractions). About 300-500 enzyme assays can be performed using this kit. The total number of assays that can be performed depends on a researcher's experimental design.

AT A GLANCE

Important Thaw all the kit components at room temperature before starting the experiment.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at $-20\text{ }^\circ\text{C}$ after preparation. Avoid repeated freeze-thaw cycles.

1. NADPH Regenerating stock solution (2X):

Make 2X NADPH Regenerating stock solution by adding the whole content of Assay Buffer II (Component B) and 500X Glucose-6-phosphate dehydrogenase (Component C) into Assay Buffer I (Component A). Mix well.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add equal volume of 2X NADPH Regenerating stock solution into the desired assay system.

Note 2.5 mL of Assay Buffer I (Component A), 2.5 mL of Assay Buffer II (Component B) and 10 μL of 500X Glucose-6-phosphate dehydrogenase (Component C) are enough for 1 plate.

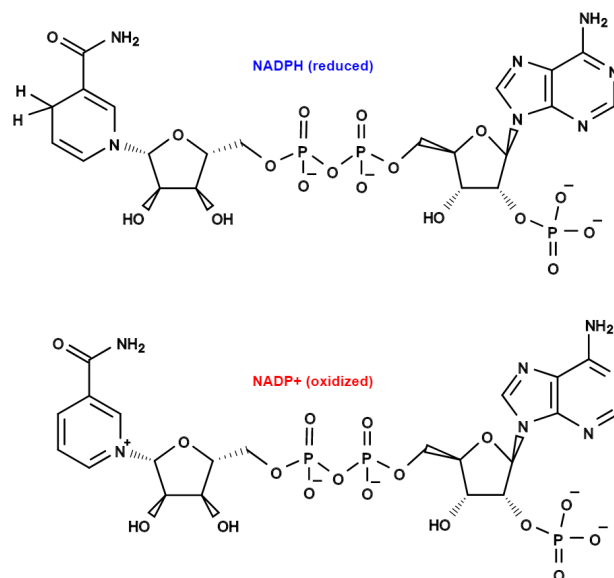
EXAMPLE DATA ANALYSIS AND FIGURES


Figure 1. Chemical structure for ReadiUse™ NADPH Regenerating Kit

DISCLAIMER

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