ReadiUse™ NADPH Regenerating Kit

Ordering Information	*Storage Conditions*
Product Number: 15265	Keep in freezer and protect from light

Introduction

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 a necessary cofactor in many xenobiotic metabolism reactions. In chloroplasts, NADP is reduced to NADPH by ferredoxin-NADP reductase in the last step of the electron chain in photosynthesis reactions. The NADPH produced is used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. Many oxidoreductases and all ligases use NADPH as coenzyme. NADPH is required for the measurement of oxidase activity catalyzed by P450s, FMOs, NADPH-P450 reductase, and many other oxidase enzymes.

AAT Bioquest's RediUse™ 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 one's experimental design.

Kit Components

Components	Amount
Component A: Assay Buffer I	1 bottle (25 mL)
Component B: Assay Buffer II	1 bottle (25 mL)
Component C: 500X Glucose-6-phosphate dehydrogenase (400 units/mL)	1 vial (100 µL)

Assay Protocol

1. Thaw Component A, Component B, and Component C at room temperature before use.
2. Make 2X NADPH Regenerating 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), and mix them well.
3. Add equal volume of 2X NADPH Regenerating Solution (from Step 2) into the desired assay system.
   Note1: Unused NADPH Regenerating solution should be aliquoted and stored at ≤ -20 °C. Keep from light and avoid repeated freeze-thaw cycles.
   Note2: 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. Aliquot and store unused Component A, B, and C at ≤ -20 °C. Keep from light and avoid *repeated freeze-thaw cycles.*

References

1. Marino D, Gonzalez EM, Frendo P, Puppo A, Arrese-Igor C. (2006) NADPH recycling systems in oxidative stressed pea nodules: a key role for the NADP(+)-dependent isocitrate dehydrogenase. Planta.
2. Diaz-Flores M, Ibanez-Hernandez MA, Galvan RE, Gutierrez M, Duran-Reyes G, Medina-Navarro R, Pascoe-Lira D, Ortega-Camarillo C, Vilar-Rojas C, Cruz M, Baiza-Gutman LA. (2006) Glucose-6 phosphate dehydrogenase activity and NADPH/NADP+ ratio in liver and pancreas are dependent on the severity of hyperglycemia in rat. Life Sci, 78, 260.
3. Pedersen A, Johansson T, Rydstrom J, Goran Karlsson B. (2005) Titration of E. coli transhydrogenase domain III with bound NADP+ or NADPH studied by NMR reveals no pH-dependent conformational change in the physiological pH range. Biochim Biophys Acta, 1707, 254.