

ReadiUse™ NADP Regenerating Kit

Ordering Information

Product Number: 15266

Storage Conditions

Keep in -20°C and protect from light

Introduction

NADP is the electron acceptor for biosynthetic reactions and dehydrogenation reactions. NADP is used for anabolic pathways, such as lipid synthesis, cholesterol synthesis and fatty acid chain elongation. It is also 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. Many oxidoreductases and all ligases use NADP/NADPH as coenzyme. NADP can also be used in the determination of amylase, creatine kinase, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase.

AAT Bioquest's ReadiUse™ NADP Regenerating Kit is designed as a flexible prepackaged NADP regenerating system to accommodate various experimental designs. It provides two ready-to-use solutions to regenerate NADP with a concentration of ~1 mM. This kit can be used for all NADP-requiring systems (such as: 12-Ketocholesterol synthesis, Ferredoxin Reductase System). 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: NADP Regenerating Buffer I	10 mL
Component B: NADP Regenerating Buffer II	10 mL
Component C: NADP Regenerating Enzyme Mix	1 vial
Component D: NADPH	2 vials

Assay Protocol

1. Thaw all the components at room temperature before use.
2. Add 0.5 mL H₂O to one NADPH vial (Component D) to make 40 X NADPH solution.
Note: One vial of NADPH is for making 10mL NADP Regenerating Solution.
3. Add 100 µL H₂O to NADP Regenerating Enzyme Mix (Component C) vial to make 400X stock solution.
Note: Aliquot and store unused Component C at ≤ -20 °C, keep from light and avoid repeated freeze-thaw cycles.
4. Prepare 2X NADP Regenerating Solution according to the following tables, protected from light.

Table 1 NADP Regenerating Solution for one 96-well plate

Components	Volume
NADP Regenerating Buffer I	5 mL
NADP Regenerating Buffer II	5 mL
NADPH	0.5 mL
NADP Regenerating Enzyme Mix	50 µL
Total volume	10.55 mL

Note 1: Activated NADP regenerating solution is not stable, and should be prepared freshly before use.

Note 2: 10 mL of NADP Regenerating Solution is enough for 1 plate. Aliquot and store unused Component A, B, C, D at ≤ -20 °C, keep from light and avoid repeated freeze-thaw cycles.

5. Add equal volume of 2X NADP Regenerating Solution (from Step 4) into the desired assay system.

References

1. Marino D, Gonzalez EM, Frendo P, Puppo A, Arrese-Igor C. (2006).
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)

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.