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ab228547 NADPH Regeneration Kit

For the regeneration of NADPH for NADPH-requiring oxidase assays.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

NADPH Regeneration Kit (ab228547) provides three 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 the experimental design.

Prepare all reagents as instructed.



Add an equal volume of 2X NADPH Regenerating Solution to the assay system.



Perform assay as normal under the usual conditions.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Item	Quantity	Storage temperature (before prep)
Assay Buffer I	25 mL	-20°C
Assay Buffer II	25 mL	-20°C
500X Glucose-6-phosphate dehydrogenase (400 units/mL)	100 µL	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Reagents and equipment normally required to run the NADPH-requiring assay of interest.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

5. Reagent Preparation

- Thaw all components at room temperature (18-25°C) prior to use.
- Briefly centrifuge small vials at low speed prior to opening.

5.1 2X NADPH Regenerating Solution

Add the entire contents of Assay Buffer II and 500X Glucose-6-phosphate dehydrogenase to Assay Buffer I. Mix thoroughly and gently.

Δ Note: Unused 2X NADPH Regenerating Solution and stored at $\leq -20^{\circ}\text{C}$ in the dark. Avoid freeze-thaw cycles.

Δ Note: 2.5 mL of Assay Buffer I, 2.5 mL of Assay Buffer II and 10 μL of 500X Glucose-6-phosphate dehydrogenase produce sufficient 2X NADPH Regenerating Solution for 1 plate. Any unused Buffer I, Buffer II and 500X Glucose-6-phosphate dehydrogenase should be stored in aliquots at $<20^{\circ}\text{C}$ in the dark. Avoid freeze-thaw cycles.

6. Assay Procedure

Equilibrate all components to the appropriate assay temperature prior to use.

- 6.1 Prepare the NADPH Regeneration Solution as directed in Section 5.
- 6.2 Prepare the NADPH-requiring assay of interest according to the usual protocol for that assay.
- 6.3 Add an equal volume of 2X NADPH Regenerating Solution to the assay of interest.
- 6.4 Run the assay of interest under the standard conditions for that assay.

7. FAQs / Troubleshooting

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

8. Notes

Technical Support

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