

Version 1a Last updated 24 August 2021

ab228548 NADP Regeneration Kit

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

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

Table of Contents

1. Overview	3
2. Materials Supplied and Storage	4
3. Materials Required, Not Supplied	5
4. General guidelines, precautions, and troubleshooting	6
5. Reagent Preparation	7
6. Assay Procedure	8
7. FAQs / Troubleshooting	9
8. Notes	10

1. Overview

NADP Regeneration Kit (ab228548) provides ready to use solutions to regenerate NADP by a simple mixing. This kit can be used for all NADP-requiring systems (e.g. 12-Ketochenodeoxycholic Acid synthesis, Ferredoxin Reductase System). 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 for 2X NADP 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.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
NADP Regenerating Buffer I	10 mL	-20°C	-20°C
NADP Regenerating Buffer II	10 mL	-20°C	-20°C
NADP Regenerating Enzyme Mix	1 vial	-20°C	-20°C
NADPH	2 Vials	-20°C	-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

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

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

5.1 NADPH

Add 0.5 mL of water to one NADPH vial to make a 40X NADPH solution.

Δ Note: One vial of NADPH is for making 10 mLs of NADP Regenerating solution.

5.2 NADP Regenerating Enzyme Mix

Add 100 μ L of water to NADP Regenerating Enzyme Mix vial to make 400X stock solution.

Δ Note: Aliquot and store unused Regenerating Enzyme Mix at -20°C, store in the dark, and avoid repeated freeze-thaw cycles.

5.3 2X NADP Regenerating solution.

Mix the reagents (prepared as in **5.1** and **5.2**) in the quantities listed in the table to produce 2X NADP Regenerating Solution. This is sufficient for 1 plate. Protect from light.

Component	Volume
NADP Regenerating Buffer I	5 mL
NADP Regenerating Buffer II	5 mL
NADPH	0.5 mL
NADP Regenerating Enzyme Mix	50 μ L
Total volume	10.55 mL

Δ Note: NADP Regenerating Solution is not stable and should be prepared fresh before each use.

Δ Note: Unused NADP Regenerating Buffer I, NADP Regenerating Buffer II, NADPH and NADP Regenerating Enzyme Mix should be aliquoted and stored at -20°C.

6. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

- 6.1** Prepare the NADP Regeneration solution as directed in Section 5.
- 6.2** Prepare the NADP-requiring assay of interest according to the usual protocol for that assay.
- 6.3** Add an equal volume of 2X NADP 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 are found at www.abcam.com/assaykitguidelines.

8. Notes

Technical Support

Copyright © 2021 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

Austria

wissenschaftlicherdienst@abcam.com | 019-288-259

France

supportscientifique@abcam.com | 01.46.94.62.96

Germany

wissenschaftlicherdienst@abcam.com | 030-896-779-154

Spain

soportecientifico@abcam.com | 91-114-65-60

Switzerland

technical@abcam.com

Deutsch: 043-501-64-24 | Français: 061-500-05-30

UK, EU and ROW

technical@abcam.com | +44(0)1223-696000

Canada

ca.technical@abcam.com | 877-749-8807

US and Latin America

us.technical@abcam.com | 888-772-2226

Asia Pacific

hk.technical@abcam.com | (852) 2603-6823

China

cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880

Japan

technical@abcam.co.jp | +81-(0)3-6231-0940

Singapore

sg.technical@abcam.com | 800 188-5244

Australia

au.technical@abcam.com | +61-(0)3-8652-1450

New Zealand

nz.technical@abc.com | +64-(0)9-909-7829