NADP/NADPH Assay Kit

Catalog No.: abx096008

Size: 96 tests

Detection Range: 0.02 µmol/L – 5.0 µmol/L

Sensitivity: 0.02 µmol/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of NADP+ and NADPH in tissue and cell samples.

Introduction

NADP (Nicotinamide adenine dinucleotide phosphate) is a cofactor for anabolic reactions which requires NADPH as a reducing agent. NADP plays important role in biosynthetic reactions including lipid and nucleic acid synthesis. The pentose phosphate pathway is the main source of NADPH in animal cells.

Abbexa's NADP/NADPH Assay Kit is a quick, convenient, and sensitive method for measuring and calculating NADP/NADPH concentration. Reduction of NADP to NADPH and further action of 1-mPMS, transfer electrons to WST-8, produces the yellow product which has an absorption maximum at 450 nm. The intensity of yellow color is proportional to the total concentration of NADP and NADPH, which can then be calculated. For the detection of NADPH, heating the sample at 60°C for 30 min, reduced WST-8 to form Formazan. Measuring the OD value at 450 nm will give the concentration of NADPH.

Kit components

1. 96-well microplate
2. Extraction solution: 60 ml x 2 vials
3. Buffer solution: 12 ml
4. Chromogenic agent: 1.2 ml x 2 vials
5. Enzyme reagent: 2 vials
6. NADPH Standard: 1 vial
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Ultrapure water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator or Sonicating water bath
7. 10 kDa ultrafiltration tube.

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml of Extraction Solution. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 12,000 x g at 4°C for 10 min. Collect the supernatant, the protein concentration in the supernatant should be determined separately (abx097193). Filter the supernatant through a 10 kDa ultrafiltration tube. Take the filtered supernatant and assay immediately.
- **Cell lysates (adherent):** After discarding the culture medium, wash the adherent cells with precooled PBS (0.01 M, pH 7.4). Scratch the cells, add 2-5 ml of PBS (0.01 M, pH 7.4) and centrifuge at 1000 × g at 4 °C for 10 min. Collect cells (4× 10^6 cells) and add Extraction agent at a ratio of 1 : 0.8. Allow the cells to stand for 10 min for lysis. Centrifuge the homogenate at 12,000 x g at 4°C for 10 min. Filter the supernatant through a 10 kDa ultrafiltration tube. Take the filtered supernatant and assay immediately.
- **Cell lysates (suspension):** Centrifuge 4× 10^6 cells at 600 × g at 4 °C for 5 min. Discard the supernatant and add 0.8 ml precooled Extraction solution. Allow the cells to stand for 10 min for lysis. Centrifuge the homogenate at 12,000 x g at 4°C for 10 min. Filter the supernatant through a 10 kDa ultrafiltration tube. Take the filtered supernatant and assay immediately.
- ***Note:*** *Extraction solution contains enzymes that can degrade NADPH. The filtration step following centrifugation is essential to prevent this.*

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Jurkat cells	1
Mark cells	1
HCT116 cells	1
293T cells	1
10% Mouse Kidney tissue homogenate	1
HeLa cells	1

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Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods at room temperature or on ice for cell lysates and tissue homogenates.
- Filtration of supernatant through a 10 KD ultrafiltration tube is essential after sample extraction and centrifugation to remove the enzymes.
- This kit shows no significant cross-reactivity with NAD+ and NADH.

2. Reagents

Bring all reagents to room temperature prior to use.

- **Enzyme reagent working solution:** Dissolve a vial of enzyme reagent with 0.12 ml of ultrapure water. Prepare immediately before carrying out the assay and mix fully. Unused Enzyme reagent working solution can be stored in dark at -20°C for up to 7 days.
- **Reaction working solution:** Dilute Enzyme reagent working solution 1:49 with Buffer solution. Prepare immediately and keep in dark before carrying out the assay and mix fully.
- **1 mmol/L NADPH standard solution:** Dissolve a vial of NADPH standard with 4.8 ml of ultrapure water. Prepare immediately before carrying out the assay and mix fully. Unused aliquoted standard solution can be stored in dark at -20°C for up to 7 days.
- **10 μmol/L NADPH standard solution:** Dilute 1 mmol/L standard solution 1:99 with Extraction solution. Prepare immediately before carrying out the assay and mix fully.

B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 μmol/L. Dilute the 10 μmol/L standard solution with Extraction solution to concentrations of 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 μmol/L. The Extraction solution itself serves as the 0 μmol/L (blank) standard.

2. Pretreatment of samples

2.1. NADPH only: Heat 0.2 ml of filtered sample supernatant at 60°C for 30 minutes to measure NADPH concentration. After heating, allow the samples to cool under running water before carrying out the assay.

2.2. Total NADPH and NADP+: Take the filtered sample supernatant directly for assay.

3. Chromogenic Reaction

3.1. Set the Standard and Sample wells.
3.2. Add 50 μl of prepared standards to the Standard wells.
3.3. Add 50 μl of sample supernatant to Sample wells.
3.4. Add 100 μl of reaction working solution to each well.
3.5. Mix fully with microplate shaker and incubate at 37°C for 10 minutes.
3.6. Add 20 μl of chromogenic agent immediately to each well.
3.7. Mix fully with microplate shaker and incubate at 37°C for 10 minutes.
3.8. Measure the OD values at 450 nm with a microplate reader.

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C. Calculation of Results

The standard curve can be plotted as the absolute OD_{450} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve (*y* = a*x* + b). The NADP and NADPH concentration of the samples can be interpolated from the standard curve.

1. Total NADP and NADPH concentration:

$$[\mathrm{NADP}]_{\mathrm{total}}(\mu\mathrm{mol/g\ protein}) = \frac{\Delta A_1 - \mathrm{b}}{\mathrm{a}} \times \frac{f}{C_P}$$

2. NADPH concentration:

$$[\mathrm{NADPH}]\ (\mu\mathrm{mol/g\ protein}) = \frac{\Delta A_2 - \mathrm{b}}{\mathrm{a}} \times \frac{f}{C_P}$$

3. NADP concentration

$$[\mathrm{NADP}]\ (\mu\mathrm{mol/g\ protein}) = [\mathrm{NADP}]_{\mathrm{total}} - [\mathrm{NADPH}]$$

4. NADP / NADPH concentration ratio

$$[\mathrm{NADP}]\ /\ [\mathrm{NADPH}]\ (\%) = \frac{[\mathrm{NADP}]_{\mathrm{total}} - [\mathrm{NADPH}]}{[\mathrm{NADPH}]} \times 100$$

where:

$[\mathrm{NADP}]_{\mathrm{total}}$	Total concentration of NADP and NADPH
$[\mathrm{NADPH}]$	Concentration of NADPH only
$[\mathrm{NADP}]$	Concentration of NADP only
$[\mathrm{NADP}]\ /\ [\mathrm{NADPH}]$	Percentage ratio of NADP to NADPH
ΔA_1	OD value of the sample for NADP and NADPH $(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}})$
ΔA_2	OD value of the sample for concentration of NADPH $(\mathrm{OD_{Sample}} - \mathrm{OD_{Blankl}})$
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
C_P	concentration of protein in sample supernatant before filtration, g protein/L

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