Technical Bulletin

NADP/NADPH Assay Kit

Catalogue Number MAK479

Product Description

Pyridine nucleotides play an important role in metabolism and there is ongoing interest in monitoring their concentration levels. Quantitative determination of $NADP^+$/NADPH has applications in research pertaining to energy transformation and redox state of cells or tissue.

Simple, direct, and automation-ready procedures for measuring $NADP^+$/NADPH concentration are useful. The NADP/NADPH Assay Kit is based on a glucose dehydrogenase cycling reaction, in which the formed NADPH reduces a formazan (MTT) reagent. The intensity of the reduced product color, measured at 565 nm, is proportionate to the $NADP^+$/NADPH concentration in the sample. This assay is highly specific for $NADP^+$/NADPH and is not interfered by NAD^+/NADH.

The linear detection range of the kit is 0.1 – 10 μM $NADP^+$/NADPH. The kit is suitable for detection of $NADP^+$/NADPH concentrations and ratios in cell or tissue extracts.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK479A
- Enzyme A 120 μL
  Catalogue Number MAK479B
- Enzyme B 120 μL
  Catalogue Number MAK479C
- MTT Solution 1.5 mL
  Catalogue Number MAK479D
- G6P 120 μL
  Catalogue Number MAK479E
- NADP Extraction Buffer 12 mL
  Catalogue Number MAK479F
- NADPH Extraction Buffer 12 mL
  Catalogue Number MAK479G
- NADP Standard (1 mM) 0.5 mL
  Catalogue Number MAK479H

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set (Catalogue Number D9063 or equivalent)
- Phosphate Buffered Saline (PBS)
- (Catalogue Number P3813 or equivalent)
- Microcentrifuge capable of RCF ≥ 14,000 × *g*
- Heat block or water bath capable of 60 °C

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate reagents to room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Notes:

a) The following substances interfere and should be avoided in sample preparation: EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween®-20 (>1%).
b) Determination of both NADP and NADPH concentrations requires extractions from two separate samples.

1. For tissues, weigh ~20 mg for each sample, wash with cold PBS.
2. For cell samples, wash cells with cold PBS and pellet ~10^5 cells for each sample.
3. Homogenize samples (either tissue or cells) in a 1.5 mL microcentrifuge tube with either 100 µL NADP Extraction Buffer for NADP determination or 100 µL NADPH Extraction Buffer for NADPH determination
4. Heat extract at 60 °C for 5 minutes.
5. To each extract, add 20 µL of Assay Buffer and 100 µL of the opposite extraction buffer to neutralize the extracts.
6. Briefly vortex and then centrifuge the samples at 14,000 × *g* for 5 minutes.
7. Transfer 40 µL of each supernatant into separate wells of a clear bottom 96-well plate for NADP/NADPH assays.

Standard Curve Preparation

At these concentrations, the standard curves for NADP and NADPH are identical. Since NADPH in solution is unstable, NADP only is provided as the standard.

1. Prepare a 10 µM NADP Standard by mixing 5 µL of 1 mM NADP Standard and 495 µL of purified water.
2. Prepare NADP standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1
Preparation of NADP Standards

Well	10 µM NADP	Purified Water	NADP (µM)
1	100 µL	-	10
2	80 µL	20 µL	8
3	60 µL	40 µL	6
4	40 µL	60 µL	4
5	30 µL	70 µL	3
6	20 µL	80 µL	2
7	10 µL	90 µL	1
8	-	100 µL	0

3. Mix well and transfer 40 µL of each Standard into separate wells of a clear 96-well plate.

Working Reagent Preparation

Note: This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

For best results allow Enzyme A and Enzyme B to come to room temperature (15-30 minutes) before preparing the Working Reagent.

1. Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 97 µL of Working Reagent according to Table 2. Fresh reconstitution is recommended.

Table 2.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	80 µL
Enzyme A	1 µL
Enzyme B	1 µL
G6P	1 µL
MTT	14 µL

2. Quickly add 80 µL of Working Reagent to all wells. Tap plate to mix briefly and thoroughly.

Measurement

1. Immediately read optical density at 565 nm for time "zero" (OD_0).
2. Incubate the plate for 30 minutes at room temperature.
3. At the 30-minute incubation point, read the plate again at 565 nm (OD_{30}). Alternatively, monitor the optical density of the plate for 30 minutes in kinetic mode at room temperature at 565 nm.

Results

1. Calculate the ΔOD values of each Standard and Sample well by subtracting OD_0 from OD_{30}.
2. Use the ΔOD values for the Standards and respective concentration to plot the standard curve and determine slope.
3. Calculate the NADP and/or NADPH concentration of the Sample using the below formula.

NADP(H) (µM) =

$$\frac{\Delta OD_{Sample} - \Delta OD_{Blank}}{Slope\ (\mu M^{-1})} \times DF$$

where:

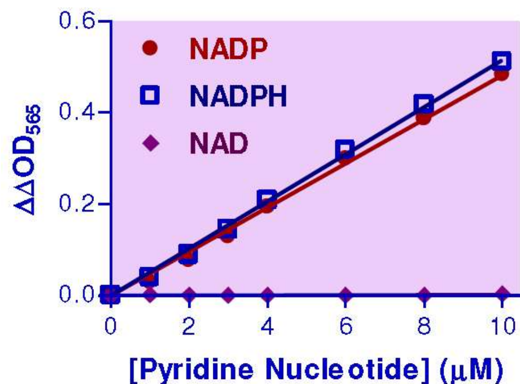
ΔOD_{Sample} = Change in OD values of Sample between zero minutes and 30 minutes.

ΔOD_{Blank} = Change in OD values of Blank (Standard #8) between zero minutes and 30 minutes.

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If the Sample ΔOD values are higher than the ΔOD value for the 10 µM Standard, dilute Sample in purified water and repeat the assay. Multiply the results by the dilution factor.

Typical NADP/NADPH Standard Curve



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

mak479pis Rev 09/22

MilliporeSigma