Product Manual

NADP+/NADPH Assay Kit (Fluorometric)

Catalog Number

MET-5031 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is an enzymatic cofactor involved in metabolic redox and cell signaling reactions. Its main function in animal metabolism is to shift electrons from one redox reaction to another. During these reactions, the coenzyme cycles between the electron donating reduced form (NADPH) and the electron accepting oxidized form ($NADP^+$). The major source of NADPH produced in animal cells is the oxidative branch of the pentose phosphate pathway (PPP). NADPH is involved with macromolecule biosynthesis by providing reducing power. These characteristics have made NADPH an important molecule in cancer cell proliferation and metabolism. NADPH is also involved in the accumulation of Reactive Oxygen Species (ROS) and protecting against its toxicity. It is also involved in anabolic pathways, such as fatty acid elongation and lipid and cholesterol synthesis. Understanding the metabolism of these cofactors has become important to developing new therapeutic methods against pathological disease states.

Cell Biolabs' $NADP^+$/NADPH Assay Kit is a simple fluorometric assay that can measure both $NADP^+$ and NADPH present in biological samples such as cell lysates or tissue extracts in a 96-well microtiter plate format. The kit is specific for $NADP^+$, NADPH, and their ratio. The kit will not detect NAD or NADH. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, $NADP^+$ standards and unknown samples. The total $NADP^+$/NADPH concentrations of unknown samples are determined by comparison with a known $NADP^+$ standard. Determination of both $NADP^+$ and NADPH requires two separate samples for quantification. $NADP^+$ and NADPH do not need to be purified from samples, but rather can be extracted individually with a simple acid or base treatment prior to performing the assay. The kit has a detection sensitivity limit of approximately 0.8 nM $NADP^+$.

Assay Principle

Cell Biolabs' $NADP^+$ /NADPH Assay Kit is a convenient quantitative tool that measures $NADP^+$ and NADPH within biological samples. The assay is based on an enzymatic cycling reaction in which $NADP^+$ is reduced to NADPH. NADPH reacts with a fluorometric probe that produces a product which can be measured with a standard 96-well fluorometric plate reader. The intensity of the product fluorescence is proportional to the $NADP^+$ and NADPH within a sample. A simple acid or base treatment will differentiate NADPH from $NADP^+$ within a sample. Samples and standards are incubated for 1-2 hours and then read at λ_{ex} = 530-570 nm/ λ_{em} = 590-600 nm (Figure 1). Samples are compared to a known concentration of $NADP^+$ standard within the 96-well microtiter plate format.

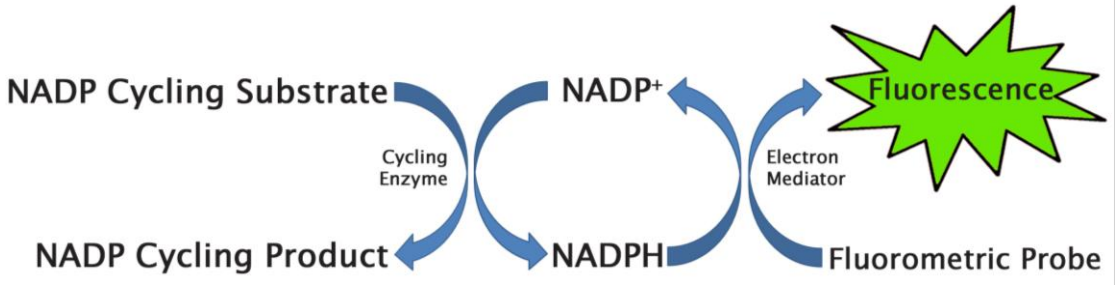

Figure 1. $NADP^+$/NADPH Cycling Assay Principle.

Related Products

1. MET-5030: NAD+/NADH Assay Kit (Fluorometric)
2. MET-5159: Pyrophosphate Assay Kit (Fluorometric)
3. MET-5023: Glycogen Assay Kit (Fluorometric)
4. MET-5164: ADP Assay Kit (Fluorometric)
5. MET-5163: ATP Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped at room temperature)

1. Fluorometric Probe (40X) (Part No. 50311C): One 150 µL amber tube
2. NADP Cycling Substrate (Part No. 50182B): One 50 µL tube
3. Assay Buffer (Part No. 50184B): One 25 mL bottle
4. Extraction Buffer (10X) (Part No. 50185B): One 10 mL bottle
5. Electron Mediator (500X) (Part No. 50312C): One 10 µL amber tube

Box 2 (shipped on blue ice packs)

1. NADP Cycling Enzyme (Part No. 50183D): One 15 µL amber tube
2. $NADP^+$ Standard (Part No. 50186D): One 50 µL amber tube of a 20 mM $NADP^+$ solution

Materials Not Supplied

1. Distilled or deionized water
2. 0.1 N NaOH
3. 0.1 N HCl
4. 10 kDa molecular weight cutoff (MWCO) centrifuge spin filter (e.g. Amicon Ultra 0.5mL)
5. 1X PBS
6. Standard 96-well fluorescence microtiter plate and/or fluorescence cell culture microplate

Storage

Upon receipt, store the NADP Cycling Enzyme and $NADP^+$ Standard at -80°C. Store the Fluorometric Probe and Electron Mediator at -20°C. Store the remaining components at 4°C.

Preparation of Reagents

- 1X Extraction Buffer: Dilute the stock 10X Extraction Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- NADP Cycling Reagent: Prepare an NADP Cycling Reagent for the number of assays being tested and just before use. Prepare by diluting the NADP Cycling Substrate 1:100, NADP Cycling Enzyme 1:400, Electron Mediator 1:500, and Fluorometric Probe 1:40 in 1X Assay Buffer. (eg. For 100 assays, combine 50 µL NADP Cycling Substrate, 12.5 µL NADP Cycling Enzyme, 10 µL Electron Mediator, and 125 µL Fluorometric Probe to 4.803 mL of 1X Assay Buffer for a 5 mL

total solution). When preparing, combine the NADP Cycling Substrate, NADP Cycling Enzyme, and Electron Mediator together first and mix prior to adding the Fluorometric Probe. Upon adding the probe, protect the solution from light to prevent the probe from oxidizing. Use the NADP Cycling Reagent immediately.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. It is highly recommended that all samples should be assayed immediately upon preparation or stored for up to 1 month at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. Samples may be diluted in deionized water as necessary before testing. Run proper controls and account for any sample dilutions. Always run a standard curve with samples.

- Tissue homogenates: Sonicate or homogenize 100 mg tissue sample in 0.5 mL cold 1X Extraction Buffer. Centrifuge at 14,000 rpm for 5 minutes at 4°C to remove insoluble material. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through. Perform dilutions in cold deionized water. Sample may be tested immediately for total $NADP^+$/NADPH quantification or extracted with acid or base to separate the cofactors. Store unused samples at -80°C for up to 1 month.

- Cell lysates: Culture cells until confluent and harvest. Centrifuge and wash cell pellet with 1X PBS. Centrifuge to pellet cells and remove wash. Resuspend cells at 1-5 x 10^6 cells/mL in 0.5 mL 1X Extraction Buffer. Homogenize or sonicate the cells on ice. Centrifuge at 14,000 rpm for 5 minutes 4°C to remove debris. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through. Perform dilutions in cold deionized water. Sample may be tested immediately for total $NADP^+$/NADPH quantification or extracted with acid or base to separate the cofactors. Store unused samples at -80°C for up to 1 month.

Note: Enzymes in tissue and cell sample lysates may deplete NADPH rapidly and affect results. Samples should be deproteinized before extracting the cofactors or using within the assay. A spin filter with a 10 kDa cutoff is recommended for efficient and clean separation.

NADPH Extraction Procedure:

To measure NADPH and destroy $NADP^+$, add 25 µL of sample to a microcentrifuge tube. Add 5 µL of 0.1 N NaOH and mix thoroughly. Incubate the tube at 80°C for 60 minutes and protected from light. Centrifuge the tube to pool all sample solution. Add 20 µL of 1X Assay Buffer to shift the pH of the sample back to neutral. Vortex to mix and centrifuge to pool sample. Sample pH should be between 6.0 and 8.0; if not, neutralize accordingly with acid or base. Keep sample on ice until assaying.

$NADP^+$ Extraction Procedure:

To measure $NADP^+$ and destroy NADPH, add 25 µL of sample to a microcentrifuge tube. Add 5 µL of 0.1 N HCl and mix thoroughly. Incubate the tube at 80°C for 60 minutes and protected from light. Centrifuge the tube to pool all sample solution. Add 20 µL of 1X Assay Buffer to shift the pH of the sample back to neutral. Vortex to mix and centrifuge to pool sample. Sample pH should be between 6.0 and 8.0; if not, neutralize accordingly with acid or base. Keep sample on ice until assaying.

Notes:

- *If testing both total $NADP^+$/NADPH and individual cofactors, dilute the total $NADP^+$/NADPH samples 1:2 with Assay buffer to maintain sample dilution consistency.*
- *Avoid samples containing SH groups like DTT, β-mercaptoethanol, or reduced gluathione.*
- *Samples should be close to neutral pH before harvesting. Samples with extremely high or low pH values could fail to yield reliable results.*

Preparation of Standard Curve

Prepare $NADP^+$ standards immediately before use.

1. First, dilute the stock 20 mM $NADP^+$ Standard solution 1:1000 in deionized water to yield a 20 µM $NADP^+$ solution (e.g., add 5 µL of the stock 20 mM $NADP^+$ Standard to 4.995 mL of deionized water). Vortex thoroughly.
2. Further dilute the 20 µM $NADP^+$ solution 1:100 in deionized water to yield a 0.2 µM (200 nM) solution (e.g., add 10 µL of the 20 µM $NADP^+$ solution to 990 µL of deionized water). Vortex thoroughly.
3. Use this 200 nM solution to prepare a series of $NADP^+$ standards according to Table 1. Do not store standard solutions.

Standard Tubes	200 nM $NADP^+$ Solution (µL)	1X Extraction Buffer (µL)	$NADP^+$ (nM)	$NADP^+$ (pmol/well)
1	250	250	100	5
2	250 of Tube #1	250	50	2.5
3	250 of Tube #2	250	25	1.25
4	250 of Tube #3	250	12.5	0.625
5	250 of Tube #4	250	6.25	0.313
6	250 of Tube #5	250	3.13	0.156
7	250 of Tube #6	250	1.56	0.078
8	250 of Tube #7	250	0.78	0.039
9	0	250	0	0

Table 1. Preparation of $NADP^+$ Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each $NADP^+$ standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of NADP Cycling Reagent to each well. Mix the well contents thoroughly and incubate for 1-2 hours at room temperature protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of $NADP^+$/NADPH within samples by comparing the sample RFU to the standard curve.

Example of Results

The following figures demonstrate typical NADP+/NADPH Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

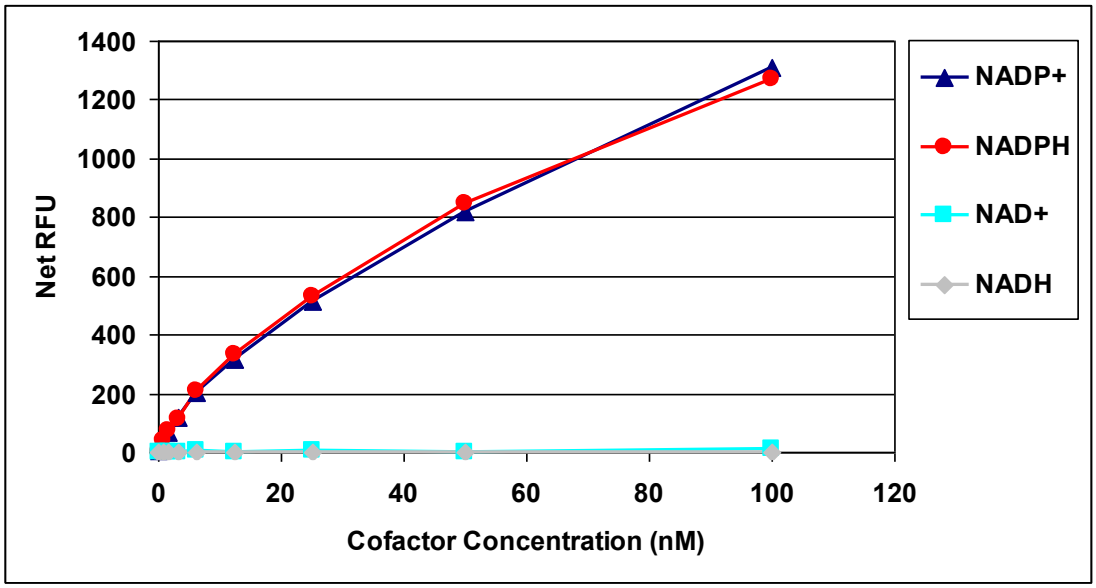

Figure 2: NADP+ Standard Curve and Specificity of Assay for NADP+ and NADPH. NADP+, NADPH, NAD+, and NADH, were tested in the NADP+/NADPH Assay Kit.

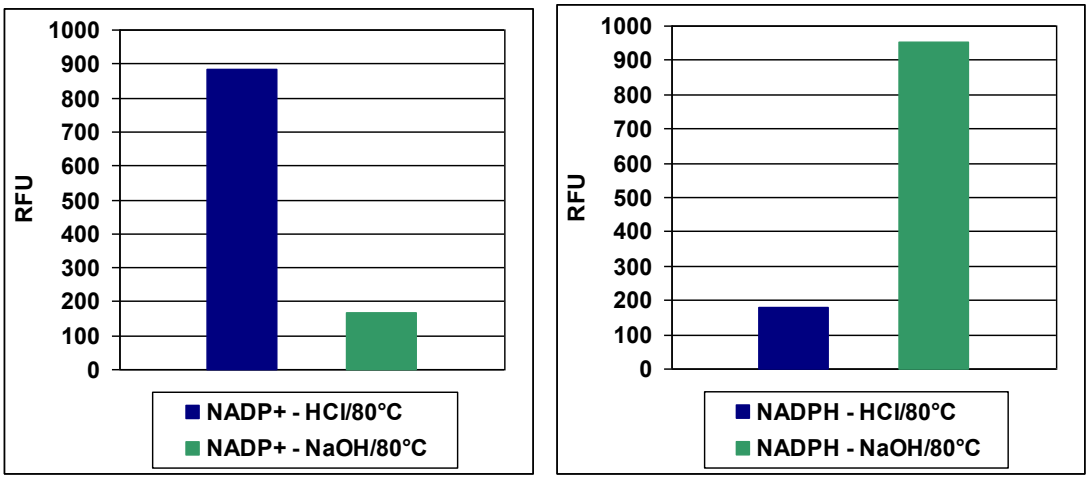

Figure 3: NADP+/NADPH Detection. NADP+ and NADPH were both tested at 100 nM with the extraction procedure. NADP+ or NADPH were incubated for 60 minutes at 80°C with 0.1N HCl or 0.1N NaOH.

Calculation of Results

1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected background fluorescence. If sample background control value is high, subtract the sample background control value from the sample reading.

2. Plot the corrected fluorescence for the $NADP^+$ standards against the final concentration of the standards from Table 1 to determine the best slope (nM^{-1}). See Figure 2 for an example standard curve.
3. Since all $NADP^+$ is converted to NADPH by the Cycling Reagent, use the standard curve to determine the total $NADP^+$/NADPH concentration in pmoles within the sample. Determine the total concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected fluorescence values for each sample. Remember to account for dilution factors.

$$\textbf{Total } NADP^+/NADPH = \left[\frac{\textbf{Sample corrected fluorescence}}{\textbf{Slope}} \right] \textbf{ x Sample dilution}$$

References

1. Bedard K, et al. (2007) *Physiol. Rev.* **87**: 245-313.
2. Berger F, et al. (2004) *Trends Biochem. Sci.* **29** (3): 111-118.
3. Hedeskov CJ, et al. (1987) *Biochem. J.* **241**: 161-167.
4. Khan JA, et al. (2007) *Expert Opin. Cell Biol.* **11** (5): 695-705.
5. Temple, MD, et al. (2005) *Trends Cell Biol.* **15** 319.
6. Veech RL, et al. (1969) *Biochem. J.* **115** (4): 609-619.

Recent Product Citations

1. Swanson, M.A. et al. (2026). A Nonketotic Hyperglycinemia Mouse Shows Wide-Ranging Biochemical Consequences of Elevated Glycine, Reduced Folate One-Carbon Charging, and Serine Deficiency. *J Inherit Metab Dis*. **49**(1):e70137. doi: 10.1002/jimd.70137.
2. Ostrakhovitch, E.A. et al. (2022). Analysis of circulating metabolites to differentiate Parkinson's disease and essential tremor. *Neurosci Lett*. **769**:136428. doi: 10.1016/j.neulet.2021.136428.

Warranty

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