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Product Information

NADP/NADPH Quantification Kit

Catalog Number **MAK038**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Nicotinamide adenine dinucleotide phosphate (NADP) is an enzymatic cofactor involved in many redox reactions where it cycles between the reduced (NADPH) and oxidized (NADP) forms. NADP is also involved in biosynthetic reactions such as lipid and nucleic acid synthesis where it functions as a reducing agent. The oxidative branch of the pentose phosphate pathway (PPP) is the major source of NADPH produced in animal cells.

The NADP/NADPH Quantification Kit provides a convenient tool for sensitive detection of the intracellular nucleotides, NADP and NADPH, and their ratio. There is no requirement to purify NADP/NADPH from samples. This assay is specific for NADP and NADPH, and does not detect NAD nor NADH. $NADP_{total}$ (NADP and NADPH) or NADPH are quantified in a colorimetric assay (450 nm).

Components

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

NADP/NADPH Extraction Buffer Catalog Number MAK038A	50 mL
NADP Cycling Buffer Catalog Number MAK038B	15 mL
NADP Cycling Enzyme Mix Catalog Number MAK038C	0.2 mL
NADPH Developer Catalog Number MAK038D	1 vl
Stop Solution Catalog Number MAK038E	1.2 mL
NADPH Standard, (MW 833.36 g/mole) Catalog Number MAK038F	166.7 µg

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter
- High-quality DMSO (Catalog Number D2650 or equivalent)

Precautions and Disclaimer

This product is 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.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Keep all enzymes on ice, protected from light, during the assay.

NADP Cycling Buffer – Allow buffer to come to room temperature before use.

NADPH Developer – Reconstitute in 1.2 mL of water. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

NADPH Standard – Reconstitute in 200 µL of pure DMSO to generate 1 nmole/µL (1 mM) solution. Mix well by pipetting. Store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on dry ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADPH Standards for Colorimetric Detection
Dilute 10 µL of the 1 nmole/µL NADPH standard with 990 µL of NADP/NADPH Extraction Buffer to generate a 10 pmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 10 pmole/µL standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add NADP/NADPH Extraction Buffer to each well to bring the volume to 50 µL.
Note: 10 pmole/µL standard solution is unstable and must be used within 4 hours.

Sample Preparation
Cell Samples – Wash cells with cold PBS. Pellet 4×10^6 cells for each assay in a microcentrifuge tube by centrifuging at 2,000 rpm for 5 minutes. Extract cells with 800 µL of NADP/NADPH Extraction Buffer. Place on ice for 10 minutes. Centrifuge the samples at 10,000 × *g* for 10 minutes to remove insoluble material. Transfer extracted NADP/NADPH supernatant into a labeled tube

Tissue Samples – Wash 50 mg of tissue with cold PBS. Homogenize by freeze/thawing the samples with 500 µL of NADP/NADPH Extraction Buffer in a microcentrifuge tube. Place on ice for 10 minutes. Centrifuge the samples at 10,000 × *g* for 10 minutes to remove insoluble material. Transfer the extracted NADP/NADPH supernatant into a labeled tube.

Note: Cell or tissue lysates may contain enzymes that consume NADPH rapidly. Samples should be deproteinized before use in assay by filtering through a 10 kDa cut-off spin filter.

Bring samples to a final volume of 50 µL with NADP/NADPH Extraction Buffer.

Assay Reaction
Detection of total NADP ($NADP_{total}$)
1a. Transfer up to 50 µL of extracted samples into labeled 96 well plate in duplicate. Bring samples to a final volume of 50 µL with NADP/NADPH Extraction Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Detection of NADPH only
1b. To detect NADPH, decompose NADP by aliquoting 200 µL of extracted samples into microcentrifuge tubes and heat to 60 °C for 30 minutes in a water bath or a heating block. Cool samples on ice. Quickly spin samples to remove any precipitates. Under these conditions, all NADP will be decomposed leaving NADPH only.

Transfer up to 50 µL of NADPH (NADP decomposed) samples into labeled 96 well plate in duplicate. Bring samples to a final volume of 50 µL with NADP/NADPH Extraction Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

2. Set up the Master Reaction Mix according to the scheme in Table 1. 100 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Master Reaction Mix
NADP Cycling Buffer	98 µL
NADP Cycling Enzyme Mix	2 µL

3. Add 100 µL of the Master Reaction Mix to each of the standard and sample wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 5 minutes at room temperature to convert NADP to NADPH.

4. Add 10 µL of NADPH developer into each well. Incubate at room temperature for 1–4 hours. Final incubation time will be dependent on color development. Measure the absorbance at 450 nm (A_{450}). The plate can be read multiple times while the color is developing.

5. The reactions can be stopped by adding 10 µL of Stop Solution into each well and mixing well. The color is stable within 48 hours in a sealed plate after addition of Stop Solution.

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) NADPH standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate NADPH standards to plot a standard curve. The amount of NADPH or $NADP_{total}$ present in the samples (all NADP in the $NADP_{total}$ reaction will have been converted to NADPH by the NADP Cycling Enzyme Mix) may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Divide the amount of $NADP_{total}$ or NADPH by either the cell number or protein concentration of the extract (ng/mL) added to the sample well. The concentration of $NADP_{total}$ or NADPH can be expressed in pmole/10^6 cells or ng/mg.

The ratio of NADP/NADPH in a sample may be determined by the following equation:

$$\text{ratio} = \frac{NADP_{total} - NADPH}{NADPH}$$

$NADP_{total}$ = Amount of total $NADP_{total}$ (NADP + NADPH) in unknown sample (pmole) from standard curve.
NADPH = Amount of NADPH in unknown sample (pmole) from standard curve.

NADPH molecular weight: 745.4 g/mole

Example:
$NADP_{total}$ amount is 87.0 pmole
NADPH amount is 43.0 pmole

The ratio of NADP/NADPH is:

$$\frac{87.0 - 43.0}{43.0} = 1.02$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Assay Buffer Ice Cold	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided.
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples.
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always prepare fresh Master Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so that readings are in the linear range

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