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Product Information

High Sensitivity NADPH Quantitation Fluorometric Assay Kit

Catalog Number **MAK216**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The reduced form of nicotinamide dinucleotide (NADPH) functions as the cofactor for many redox enzymes and contributes to reductive biosynthetic pathways. NADPH is mainly produced from glucose through the oxidative pentose phosphate pathway.[1,2]

The High Sensitivity NADPH Quantification Fluorometric Assay Kit is suitable for quantifying NADPH (ranging from 2–10 pmole/well) in tissues, cells, and mitochondria. The kit detects intracellular NAPDH in the enzyme recycling reaction and does not require purified NADPH from the samples. NADPH is determined by measuring a fluorescent product (λ_{ex} = 535/ λ_{em} = 587 nm) proportional to the amount of NADPH present. .

Components

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

NADPH Extraction Buffer Catalog Number MAK216A	50 mL
NADPH Cycling Buffer Catalog Number MAK216B	15 mL
High-Sensitivity Probe Catalog Number MAK216C	0.4 mL
NADPH Cycling Enzyme Mix Catalog Number MAK216D	0.2 mL
NADPH Standard Catalog Number MAK216E	1 vl

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader
- Dimethyl Sulfoxide, DMSO (Catalog Number D2650 or equivalent)
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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 the vials at low speed before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

NADPH Extraction Buffer and NADPH Cycling Buffer – Store at –20 °C. Warm to room temperature before use.

High-Sensitivity Probe – Store at –20 °C. Warm to room temperature before use.

NADPH Cycling Enzyme Mix – Aliquot and store at –70 °C. Use within 2 months. Keep on ice while in use.

NADPH Standard – Reconstitute with 200 µL of DMSO to generate a 1 mM (1 nmole/µL) NADPH Standard Solution. Mix by pipetting. Aliquot and store at –20 °C. Use within 2 months. Keep on ice while in use.

Storage/Stability

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

Procedure
All samples and standards should be run in duplicate.

NADPH Standards
Dilute 10 µL of the 1 mM NADPH Standard with 990 µL of NADPH Extraction Buffer and mix well to make a 10 µM (10 pmole/µL) NADPH Standard Solution. Further dilute 50 µL of the 10 µM NADPH Standard with 450 µL of NADPH Extraction Buffer and mix well to make a 1 µM (1 pmole/µL) NADPH Standard Solution. Prepare diluted Standard Solution within 4 hours of use. Add 0, 2, 4, 6, 8, and 10 µL of the 1 µM (1 pmole/µL) NADPH Standard Solution into a 96 well plate generating 0 (blank), 2, 4, 6, 8, and 10 pmole/well standards. Add NADPH Extraction Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Homogenize 10 mg of sample tissue with 200 µL of ice cold NADPH Extraction Buffer. Keep on ice for 10 minutes. Centrifuge the samples at 10,000 × *g* for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.

Add 1–50 µL of the supernatant sample to duplicate tubes. Bring samples to a final volume of 80 µL using NADPH Extraction Buffer.

NADP must be decomposed in order to detect NADPH. Incubate the samples at 60 °C for 30 minutes to decompose NADP. Cool the samples on ice. Centrifuge briefly and add 50 µL of the sample into a 96 well plate.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the NADPH Cycling Enzyme Mix. The Sample Blank readings can then be subtracted from the sample readings.

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

Table 1.
Reaction Mixes

Reagent	Standards and Samples	Sample Blank
NADPH Cycling Buffer	97 µL	99 µL
NADPH Cycling Enzyme Mix	2 µL	–
High-Sensitivity Probe	1 µL	1 µL

2. Add 100 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at room temperature for 60 minutes.

4. Measure the fluorescence (λ_{ex} = 535/ λ_{em} = 587 nm) in a microplate reader.

Results

Calculations

Correct for the background by subtracting the measurement obtained for the 0 (blank) NADPH Standard from that of the standards and samples. 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.

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

Subtract the Sample Blank value from the Sample value to obtain the corrected measurement. Using the corrected measurement, determine the amount of NADPH (pmole/well) generated by the assay (S_a).

Concentration of NADPH

$C = S_a/S_v$

where:

S_a = Amount of NADPH in unknown sample well (pmole) from standard curve

S_v = Sample volume (μL) added into the well

C = Concentration of NADPH in sample (pmole/μL)

Sample Calculation

Amount of NADPH (S_a) = 5.84 pmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of NADPH in sample decomposition tube:

$$5.84 \text{ pmole}/50\ \mu\text{L} = 0.117 \text{ pmole}/\mu\text{L}$$

Molecular weight of NADPH: 744.41 g/mole

$$0.117 \text{ pmole}/\mu\text{L} \times 744.41 \text{ pg/pmole} = 87.1 \text{ pg}/\mu\text{L}$$

References

1. Jiang, P. et al., Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. Nature, **493**, 689–693 (2013).
2. Son, J. et al., Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature, **496**, 101–105 (2013).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	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 Fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	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 needed to use 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	Prepare fresh Reaction Mixes before each 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 Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes 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 readings are in the linear range

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