ab287848 – PicoProbe NADPH Fluorometric Assay Kit

For the quantitative measurement Measurement of NADPH in various tissues/cells
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287848

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability
Store kit at –20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Extraction Buffer II/NADPH Extraction Buffer	50 mL	–20°C
NADP Cycling Buffer/NADPH Cycling Buffer	15 mL	–20°C
PicoProbe I/PicoProbe™	0.4 mL	–20°C
NADPH Cycling Enzyme Mix	0.2 mL	–20°C
NADPH Standard (Lyophilized)	1 Vial	–20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (Fluorescence reader)

Reagent Preparation

- Read the entire protocol before performing the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

PicoProbe I/PicoProbe™: Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

NADPH Cycling Enzyme Mix: Ready to use as supplied. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.

NADPH Standard: Reconstitute with 200 µl DMSO to generate 1 mM (1 nmol/µl) NADPH Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.

Assay Protocol

- Warm Extraction Buffer II/NADPH Extraction Buffer & NADP Cycling Buffer/NADPH Cycling Buffer to room temperature before use.
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Sample Preparation

- Rapidly homogenize tissue (10 mg) or cells (1 x 106) on ice with 200 µl ice cold Extraction Buffer II/NADPH Extraction Buffer. Keep on ice for 10 min.
- Centrifuge at 10,000 X g for 5 min. Collect the supernatant.
- Add 1-50 µl sample into an eppendorf tube and bring the volume to 80 µl with Extraction Buffer II/NADPH Extraction Buffer.

Δ Notes:

a) For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

b) For samples having high background, prepare parallel sample well(s) as sample background control.

NADP Decomposition

- To detect NADPH, the NADP needs to be decomposed before the reaction. Keep samples at 600C for 30 min. to completely decompose the NADP. Cool samples on ice.
- Centrifuge briefly and transfer 50 µl of samples into a 96-well plate.

Standard Curve Preparation

- Dilute NADPH Standard to 10 µM (10 pmol/µl) by adding 10 µl of 1 mM NADPH Standard to 990 µl Extraction Buffer II/NADPH Extraction Buffer, mix well.
- Dilute 10 µM NADPH Standard further to 1 µM (1 pmol/µl) by adding 50 µl of 10 µM NADPH to 450 µl Extraction Buffer II/NADPH Extraction Buffer, mix well.
- Add 0, 2, 4, 6, 8 & 10 µl of diluted 1 µM NADPH Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8, and 10 pmol/well NADPH Standards.
- Adjust the volume to 50 µl per well with Extraction Buffer II/NADPH Extraction Buffer.

  Δ Note: *Prepare working solution of NADPH Standard just before use (use within 4 hrs). Don't store the diluted Standard.*

Reaction Mix:

- Prepare enough reaction mix for the number of assays (Standards, samples and background controls) to be performed. For each well, prepare 100 µl Reaction Mix containing:

	Sample Reaction Mix	Background Control Mix
NADP Cycling Buffer/NADPH Cycling Buffer	97 µL	99 µL
NADPH Cycling Enzyme Mix	2 µL	-
PicoProbe I/PicoProbe™	1 µL	1 µL

- Add 100 µl of Reaction Mix to each well containing the Standards & samples, mix well.

  Δ Note: *For samples having high background, add 100 µl of Background Control Mix to sample background control well(s). Mix well.*

Measurement

- Incubate the reaction for 60 min. at room temperature. Measure fluorescence (Ex/Em = 535/587 nm).

Calculation

Subtract 0 Standard reading from all readings. Plot the NADPH Standard Curve. If the sample background control reading is significant, subtract the background control reading from sample readings. Apply the corrected sample reading to the Standard Curve to get B pmol of NADPH in the sample wells.

Sample NADPH Concentration (C) = B/V X Dilution Factor = pmol/µL = nmol/mL = µM

Where:
B is the NADPH amount from the Standard Curve (pmol)
V is the sample volume used in the reaction well (µL)

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For technical support contact information, visit: www.abcam.com/contactus