

Product datasheet

NADP/NADPH Assay Kit ab65349

★★★★★ [1 Abreviews](#) [161 References](#) [6 Images](#)

Overview

Product name	NADP/NADPH Assay Kit
Detection method	Colorimetric
Sample type	Tissue Extracts, Cell Lysate
Assay type	Quantitative
Assay time	2h 00m
Product overview	<p>NADP/NADPH Assay Kit (ab65349) provides a convenient tool for sensitive detection of the intracellular nucleotides: NADP, NADPH and their ratio. Assays of nicotinamide nucleotides are of continual interest in the studies of energy transforming and redox state of cells or tissue.</p> <p>The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction. The assay does not recognize NAD⁺/NADH. There is no need to purify NADP/NADPH from the sample mix. The enzyme cycling reaction significantly increases detection sensitivity. Results can be quantified using a plate reader at OD450nm.</p> <p>NADP / NADPH assay protocol summary:</p> <ul style="list-style-type: none">- extract samples from cells / tissues with extraction buffer and deproteinize with spin column- for NADPH measurement, heat samples to 60°C for 30 min to decompose NAD⁺, cool on ice (this step not necessary for measurement of total NADP⁺/NADPH)- add samples and standards to wells- add reaction mix and incubate for 5 min at room temp to convert NADP to NADPH- add NADPH developer and incubate for 1-4 hrs while reaction cycles- analyze with microplate reader multiple times during the 1-4 hr incubation- reaction can be stopped with stop solution
Notes	<p>This product is manufactured by BioVision, an Abcam company and was previously called K347 NADP/NADPH Quantitation Colorimetric Kit. K347-100 is the same size as the 100 test size of ab65349.</p> <p>If you would like to use a fluorometric reading, please refer to NADP/NADPH Assay Kit (Fluorometric) (ab176724).</p>
Platform	Microplate reader

Properties

Storage instructions

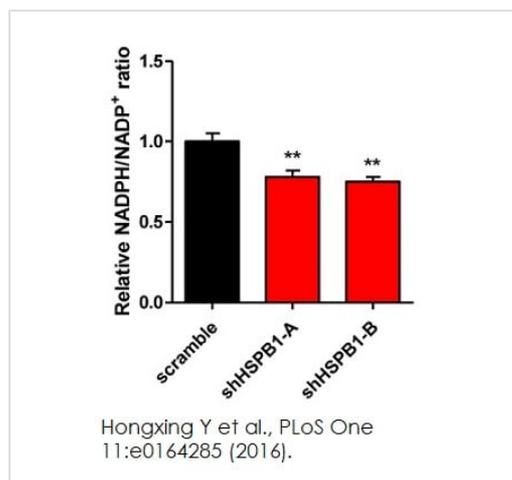
Store at -20°C. Please refer to protocols.

Components	100 tests
Developer Solution II	1 vial
Extraction Buffer II	1 x 50ml
NADP Cycling Buffer	1 x 15ml
NADP Cycling Enzyme Mix	1 x 0.2ml
NADPH Standard	1 x 166.7µg
Stop Solution II	1 x 1.2ml

Relevance

NADP (Nicotinamide adenine dinucleotide phosphate) is a coenzyme composed of ribosylnicotinamide 5-phosphate (NMN) coupled by pyrophosphate linkage to the 5-phosphate adenosine 2,5-biphosphate. It serves as an electron carrier in a number of reactions, being alternately oxidised (NADP⁺) and reduced (NADPH). The oxidative phase of the pentose phosphate pathway is the major source of NADPH in cells, producing approximately 60% of the NADPH required. NADPH provides the reducing equivalents for biosynthetic reactions and the oxidation-reduction involved in protecting against the toxicity of ROS, allowing the regeneration of GSH. NADPH is also used for anabolic pathways, such as lipid synthesis, cholesterol synthesis and fatty acid chain elongation.

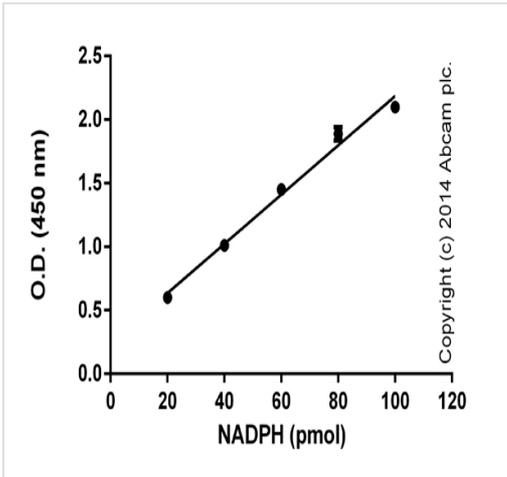
Images



NAPDH/NADP⁺ ratio was determined using ab65349 in stable HSPB1- knockdown U87 cells.

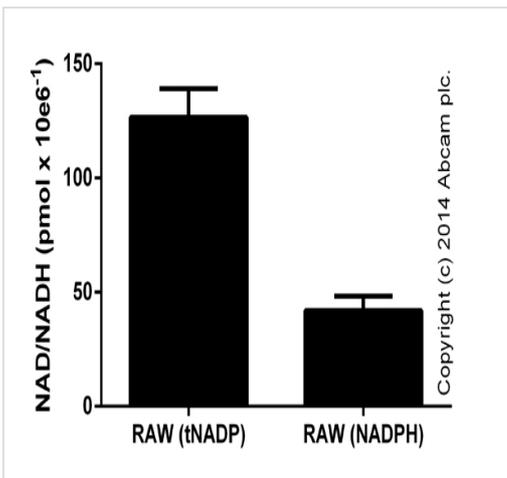
Functional studies- ab65349

Image from Hongxing Y et al., PLoS One 11(10), Fig 2a.
Doi:10.1371/journal.pone.0164285 Reproduced under the Creative Commons license
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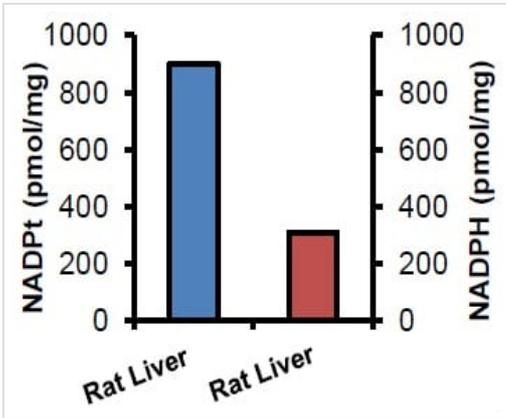
Standard curve with background signal subtracted (duplicates; +/- SD).

Functional assay: NADP/NADPH Assay Kit
(ab65349)



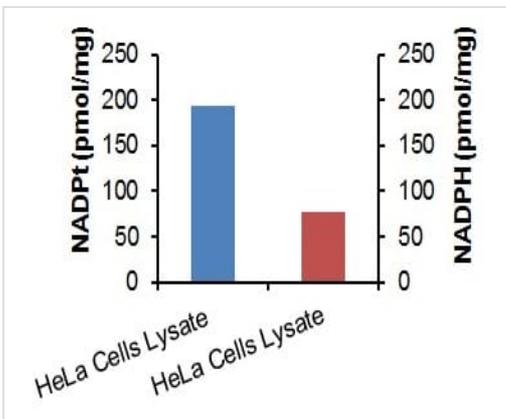
Total NADP and NADPH (tNADP) or NADPH alone measured in RAW cell lysates (duplicates +/- SD).

Functional assay: NADP/NADPH Assay Kit
(ab65349)



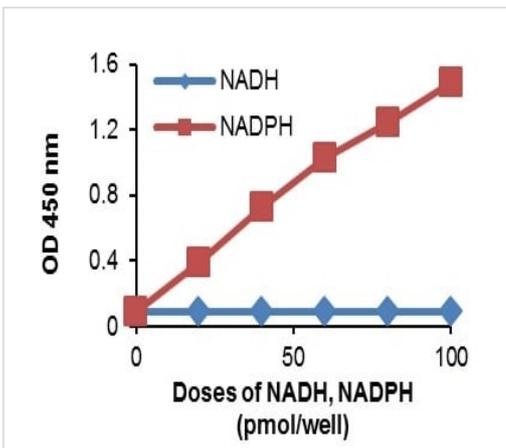
Functional Studies - NADP/NADPH Assay Kit
(ab65349)

Measurement of NADPt and NADPH in rat liver lysate (20 µg).
Assays were performed using the kit protocol.



Functional Assay - ab65349

Measurement of NADPt and NADPH in HeLa cell lysate (80 µg).
Assays were performed using the kit protocol.



Typical Standard Curve - ab65349

Typical standard curve for ab65349.

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