# abcam

# Product datasheet

# NADP/NADPH Assay Kit ab65349

★★★★★ 1 Abreviews 161 References 画像数 6

#### 製品の概要

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製品名 NADP/NADPH Assay Kit

検出方法 Colorimetric

サンプルの種類 Tissue Extracts, Cell Lysate

アッセイタイプ Quantitative

**全工程の試験時間** 2h 00m

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.

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.

NADP / NADPH assay protocol summary:

- 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<sup>+</sup>, cool on ice (this step not necessary for measurement of total NADP<sup>+</sup>/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

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.

If you would like to use a fluorometric reading, please refer to NADP/NADPH Assay Kit

(Fluorometric) (ab176724).

試験プラットフォーム Microplate reader

製品の特性

特記事項

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#### 保存方法

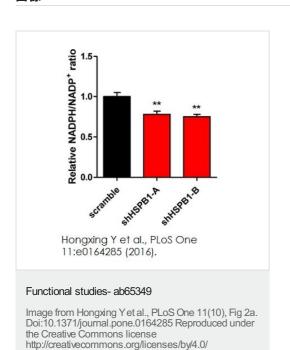
Store at -20°C. Please refer to protocols.

内容	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

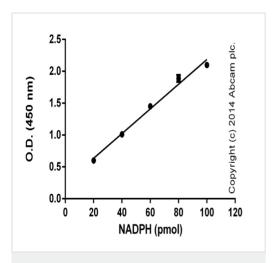
#### 関連性

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 approxiamtely 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.

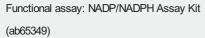
## 画像

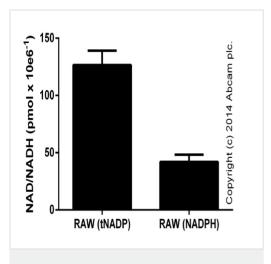


NAPDH/NADP<sup>+</sup> ratio was determined using ab65349 in stable HSPB1- knockdown U87 cells.



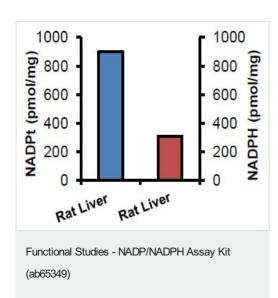
Standard curve with background signal subtracted (duplicates; +/-SD).



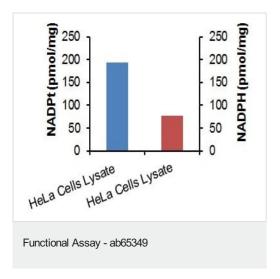


Total NADP and NADPH (tNADP) or NADPH alone measured in RAW cell lysates (duplicates +/- SD).

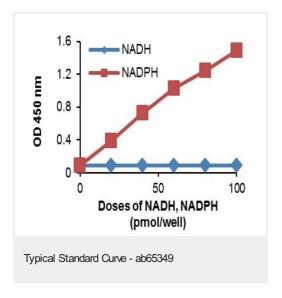
Functional assay: NADP/NADPH Assay Kit (ab65349)



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



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



Typical standard curve for ab65349.

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