

NAD/NADH Assay Kit (Colorimetric) ab65348

[245 References](#) [5 Images](#)

Overview

Product name	NAD/NADH Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Urine, Serum, Cell Lysate, Tissue Lysate
Assay type	Quantitative
Range	400 nM - 2000 nM
Assay time	2h 00m
Product overview	NAD/NADH Assay Kit (Colorimetric) ab65348 provides a convenient and sensitive tool to quantify NAD ⁺ and NADH, and measure their ratio, in samples from mammals and other species.

The NAD cycling enzyme mix in the kit specifically acts on NADH/NAD in an enzyme cycling reaction which significantly increases sensitivity and specificity. There is no requirement to purify NADH/NAD from samples.

The levels of both NADt (total NAD⁺ and NADH) and NADH can be easily measured; the level of NAD⁺ can be easily calculated by subtracting NADH from NADt. The assay is read by absorbance at 450 nm.

NAD / NADH assay protocol summary:

- extract samples from cells / tissues with extraction buffer and deproteinize with spin column
- for NADH measurement, heat samples to 60°C for 30 min to decompose NAD⁺, cool on ice (this step not necessary for measurement of total NAD⁺/NADH)
- add samples and standards to wells
- add reaction mix and incubate for 5 min at room temp to convert NAD to NADH
- add NADH 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 This product is manufactured by BioVision, an Abcam company and was previously called K337 NAD/NADH Quantitation Colorimetric Kit. K337-100 is the same size as the 100 test size of ab65348.

This assay specifically detects NAD and NADH, but not NADP nor NADPH.

If you would like to use a fluorometric reading, please refer to NAD/NADH Assay Kit (Fluorometric) ([ab176723](#)).

NAD/NADH Assay kit **ab221821** uses an alternative assay method that relies on purification of NAD and NADH from samples and may be more sensitive in some samples.

Review our [Metabolism Assay Guide](#) to learn about assays for metabolites, metabolic enzymes, mitochondrial function, and oxidative stress, and also about how to assay metabolic function in live cells using your plate reader.

How other researchers have used NAD/NADH Assay Kit ab65348

This NAD/NADH assay kit has been used in publications in a variety of sample types, including:

- Human: primary blood mononuclear cells¹, epithelial ovarian cancer cells², Jurkat cells³
- Mouse: cell culture lysates⁴, cardiomyocyte cell culture lysates⁵, liver⁶, liver and muscle⁷, primary hepatocyte cell cultures⁸, aorta tissue⁹
- Rat: brain tissue¹⁰
- Locust: thoracic muscle¹¹
- Bacteria: *Z. mobilis*¹², *E. coli*¹³

References: 1 - Castro-Marrero J et al 2015; 2 - Zhu J et al 2019, Xia H et al 2015; 3 - Miller TW et al 2015; 4 - Mekala NK et al 2019, Ling S et al 2017; 5 - Zhang D et al 2019; 6 - Shao D et al 2017, Mukherji A et al 2015, Yu JH et al 2016; 7 - Karandrea S et al 2017; 8 - Traboulsi H et al 2014; 9 - Liu Y et al 2016; 10 - Rao G et al 2016; 11 - Ding D et al 2018; 12 - Wu B et al 2019; 13 - Long YMet al 2017

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate reader

Properties

Storage instructions

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

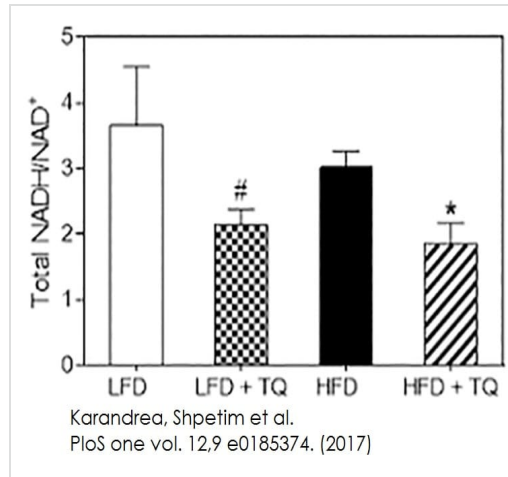
Components	100 tests	2000 tests
Cycling Buffer I	1 x 15ml	20 x 15ml
NAD Cycling Enzyme Mix	1 vial	20 vials
Developer Solution II	1 vial	20 vials
NADH Standard II	1 vial	20 vials
Extraction Buffer II	1 x 50ml	20 x 50ml
Stop Solution II	1 x 1.2ml	20 x 1.2ml

Relevance

NAD (Nicotinamide adenine dinucleotide) is a coenzyme in metabolic redox reactions, a precursor for several cell signaling molecules, and a substrate for protein posttranslational modifications. NAD is a dinucleotide, consisting of two nucleotides joined through their phosphate groups: with one nucleotide containing an adenosine ring, and the other containing nicotinamide. In metabolism, NAD is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is therefore found in two forms in cells: NAD is an oxidizing agent – it

accepts electrons from other molecules and becomes reduced, forming NADH, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD. However, it is also used in other cellular processes, the most notable one being a substrate of enzymes that add or remove chemical groups from proteins in posttranslational modifications.

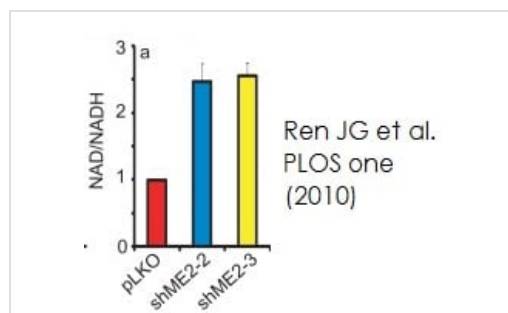
Images



NADH/NAD⁺ ratio in soleus muscle measured using ab65348. p ? 0.05 when comparing (+) HFD and LFD, (*) HFD + TQ and HFD, and (#) LFD and LFD + TQ using independent t-tests. Results are means \pm SEM (n = 8-10 mice per treatment group). LFD: low fat diet, HFD: high fat diet, TQ: thymoquinone.

Functional Studies - NAD/NADH Assay Kit
(Colorimetric) (ab65348)

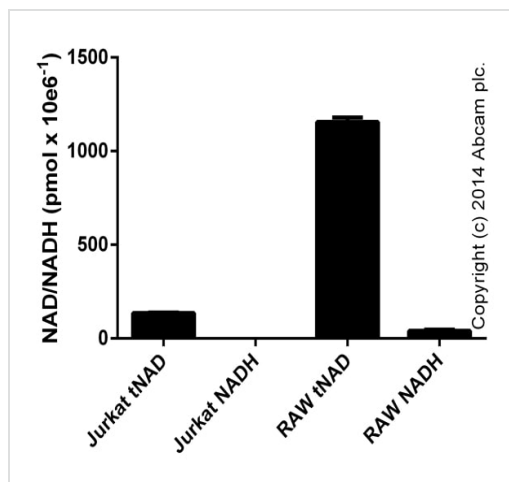
Karandrea, Shpetim et al., PLoS one vol. 12,9 e0185374., Fig 6, doi:10.1371/journal.pone.0185374



NAD/NADH was measured in K562 ME2 knockdown cells(pLKO - empty vector; shME2-2 & shME2-3 - two selected knockdown clones). Data are expressed as mean \pm SD, n=3. NAD/NADH Ratio is calculated as described in the product protocol. *Image obtained from Ren JG et al; PLOS one, 2010; 5(9): e12520 (DOI:10.1371/journal.pone.0012520)*

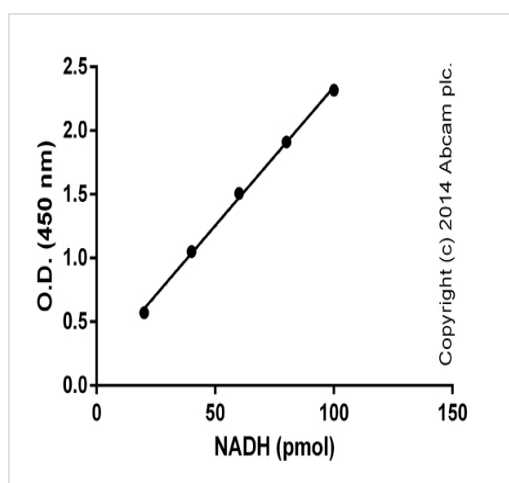
Functional Studies - NAD/NADH Assay Kit
(ab65348)

Ren JG et al., PLoS One, 5, , 2010 Reproduced under the Creative Commons license
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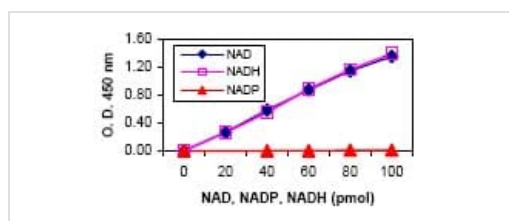
Functional assay: NAD/NADH Assay Kit (ab65348)

NAD and NADH (tNAD) or NADH alone measured cell lysates. 5e6 cells were lysed in 1 mL, spin filtered, and tested neat or 1/5 (duplicates +/- SD).



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

Functional assay: NAD/NADH Assay Kit (ab65348)



NADH Standard calibration curve. Quantification of NAD (diamond) and NADH (open square) following product protocol and using NADH standard provided in the kit. No NADP (triangle) was detected in this reaction. Standard curve range: 20-100 pmol.

Functional Studies - NAD+/NADH Assay Kit (ab65348)

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