abcam

Product datasheet

NAD/NADH Assay Kit II (colorimetric) ab221821

5 Images

Overview

Product name	NAD/NADH Assay Kit II (colorimetric)
Detection method	Colorimetric
Sample type	Cell Lysate
Assay type	Quantitative
Sensitivity	62.5 nM
Species reactivity	**Reacts with:** Mammals
Product overview	NAD/NADH Assay Kit II (Colorimetric) (ab221821) provides a sensitive and robust method to measure NAD^+, NADH and their ratio in mammalian cell lysates. The assay is based on an ADH and diaphorase coupled-reaction that convers WST-1 to WST-1 formazan, which can be easily detected at OD 450 nm. As the reaction is not stopped, it is necessary to monitor the absorbance increase of WST-1 formazan at regular intervals after the reaction is initiated to determine the reaction velocity. This assay requires purification of NAD^+ and NADH from the cell lysates, which raises the efficiency of the reaction and increases the detection sensitivity.
Notes	Nicotinamide nucleotides are key players in the energy and oxidation-reduction reactions of a cells. Nicotinamide adenine dinucleotide (NAD) exists in two forms, an oxidized form, NAD+, and a reduced form, NADH. NAD functions as a cofactor in the vast majority of cellular redox reactions, carrying reducing equivalents from one reaction to another. Therefore, maintaining appropriate levels of NAD is essential for maintaining normal cellular respiratory function. There are two major pathways in NAD biosynthesis. The *de novo* pathway is maintained by the rate-limiting enzyme nicotinamide phosphoribosyltransferase (NAMPT), whereas the savage pathway recycles degraded NAD products such as nicotinamide. Studies have shown that cytosolic NAD^+ concentrations range from 300 nM in mammalian cells to 2 mM in yeast. Depletion of NAD in cells is a major cause of cell death. The importance of NAD function in modulating cellular redox status and controlling signaling and transcriptional events makes NAD an important cofactor when investigating normal cellular function.
Platform	Microplate reader

Properties

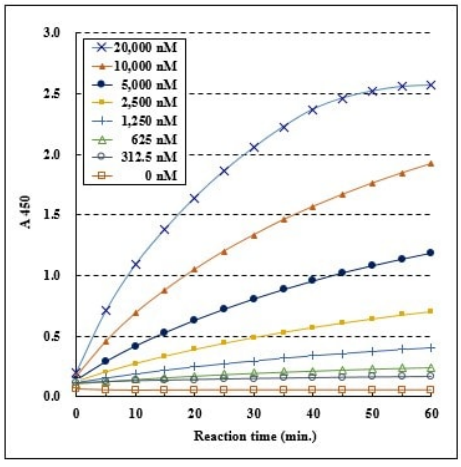
Storage instructions Store at -80°C. Please refer to protocols.

Components	100 tests
20X Standard Dilution Buffer	1 x 1ml
400 µM NADH	1 x 200µl
ADH	1 x 500µl
Diaphorase	1 x 500µl
EtOH Solution	1 x 500µl
NAD+/NADH Assay Buffer (20X)	1 x 1ml
WST-1	1 x 500µl

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



NAD/NADH Assay Kit II (Colorimetric) (ab221821) Time curve kinetic curve of NADH standards

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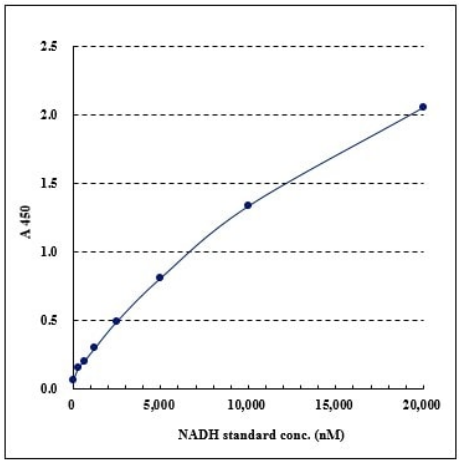

NAD/NADH Assay Kit II (Colorimetric) (ab221821) Typical NADH standard curve

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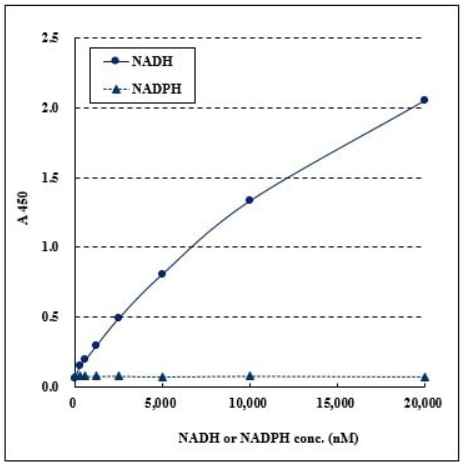
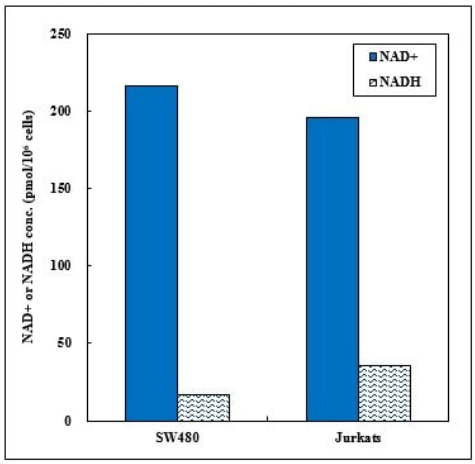


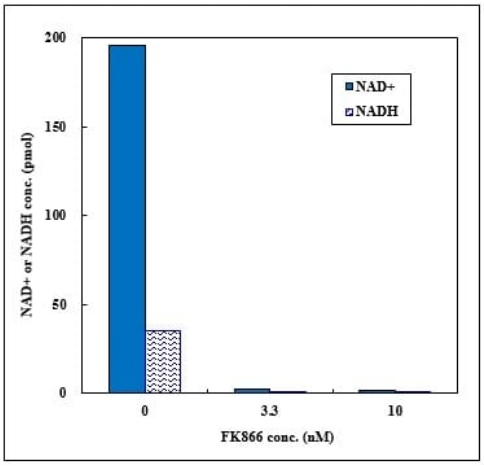
NAD/NADH Assay Kit II (Colorimetric) (ab221821) Specific detection of NADH. The assay shows specificity for NADH. No response seen from NADPH present in the reaction well. (As low as 312.5 nM of NADH can be detected with 30 minutes incubation time (n=2), there is no response to NADPH.)

NAD/NADH Assay Kit II (Colorimetric) (ab221821) Specific detection of NADH



NAD/NADH Assay Kit II (Colorimetric) (ab221821) NAD+ and NADH concentrations in cell extracts

NAD/NADH Assay Kit II (Colorimetric) (ab221821) NAD^+ (solid bar) and NADH (blue and white bar) concentrations in cell extracts from SW480 (human colon cancer) and Jurkat (human T lymphocyte) cells.



NAD/NADH Assay Kit II (Colorimetric) (ab221821) NAD+ and NADH concentrations in Jurkatccell extracts treated with increasing concentrations of FK866

NAD/NADH Assay Kit II (Colorimetric) (ab221821) NAD^+ (solid bar) and NADH (blue and white bar) concentrations in Jurkat cell extracts treated with increasing concentrations of FK866, a NAMPT specific inhibitor.

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