abcam

Product datasheet

Total NAD and NADH Assay Kit (Colorimetric) ab186032

4 Images

Overview

Product name	Total NAD and NADH Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Urine, Serum, Plasma, Cell Lysate, Tissue Lysate
Assay type	Quantitative
Sensitivity	> 0.1 µM
Range	0.078 µM - 5 µM
Assay time	2h 00m
Product overview	Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are two important cofactors found in cells. NADH is the reduced form of NAD+. NAD forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. The traditional NAD/NADH and NADP/NADPH assays are based on monitoring the changes in NADH or NADPH absorption at 340 nm. The short UV wavelength of NAD/NADH and NADP/NADPH assays makes the traditional methods suffer low sensitivity and high interference. Abcam's Colorimetric total NAD/NADH Assay Kit (ab186032) provides a convenient method for detecting total NAD and NADH. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from the sample mix. The enzyme cycling reaction significantly increases detection sensitivity. The NADH probe is a chromogenic sensor that has its maximum absorbance at 460 nm upon NADH reduction. The absorption of the NADH probe is directly proportional to the concentration of NADH in the solution. The Colorimetric total NAD and NADH Assay Kit provides a sensitive assay to detect as little as 0.1 µM total NAD/NADH in a 100 µL assay volume.
Notes	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

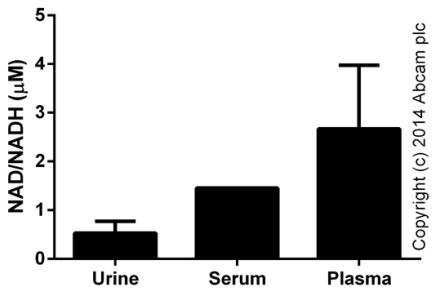
Storage instructions Store at -20°C. Please refer to protocols.

Components	
NADH Probe Buffer	1 x 16ml
NAD/NADH Recycling Enzyme Mixture	2 x 1vial
NADH Standard	1 x 142µg
Lysis Buffer	1 x 10ml
NADH Probe	1 x 4ml

Relevance

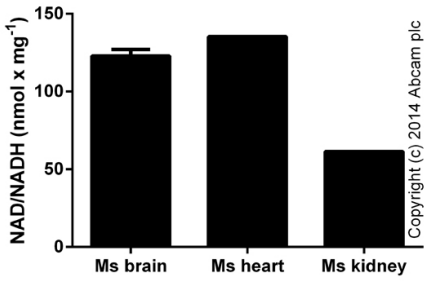
NAD (Nicotinamide adenine dinucleotide) is a coenzyme in metabolic redox reactions, a precursor for several cell signaling molecules, and a substrate for protein posttranslational modifications. NAD is a dinucleotide, consisting of two nucleotides joined through their phosphate groups: with one nucleotide 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



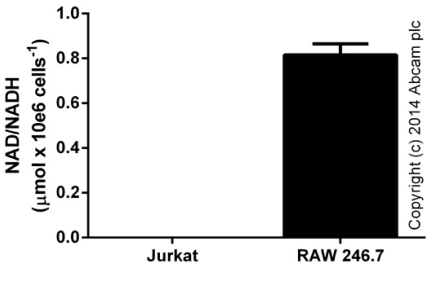
Functional assay: Total NAD and NADH Assay Kit (Colorimetric) (ab186032)

NAD/NADH measured in undiluted biological fluids (duplicates +/- SD).



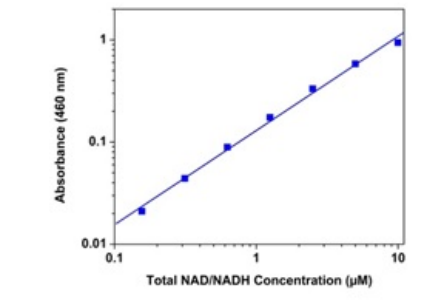
Functional assay: Total NAD and NADH Assay Kit (Colorimetric) (ab186032)

NAD/NADH measured in mouse tissue lysates (1-20 mg protein x mL^{-1} tested, expressed as per mg of extracted protein; duplicates +/- SD).



Functional assay: Total NAD and NADH Assay Kit (Colorimetric) (ab186032)

NAD/NADH measured cell lysates (Jurkat sample below level of detection; duplicates +/- SD).



NADH standard curve

NADH dose response was measured with total NAD and NADH Assay Kit (Colorimetric) in a 96-well white/clear bottom plate using a microplate reader. As low as 0.1 µM of NADH can be detected with 1 hour incubation (n=3) with absorbance measurement at 460nm. The absorbance in blank wells (with the PBS buffer only) is used as a control, and is subtracted from the values for those wells with the NADH reactions.

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