Datasheet for ABIN1000284

NAD/NADH Assay Kit

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Overview

Quantity:	100 tests
Target:	NAD/NADH
Application:	Biochemical Assay (BCA)

Product Details

Sample Type:	Cell Extracts
Specificity:	0.05 µM
Characteristics:	Sensitive and accurate. Detection limit 0.05 µM, linearity up to 10 µM NAD + /NADH in 96-well plate assay. Convenient. The procedure involves adding a single working reagent, and reading the optical density at time zero and 15 min at room temperature. No 37°C heater is required. High-throughput. Can be readily automated as a high-throughput 96- well plate assay for thousands of samples per day.
Components:	Assay Buffer: 10 mL. Lactate: 1.5 mL. MTT Solution: 1.5 mL. Enzyme A: 120 µL. NAD Standard: 0.5 mL 1 mM. Enzyme B: 120 µL. NAD/NADH Extraction Buffers: each 12 mL.
Material not included:	Pipetting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Target Details

Target:	NAD/NADH
Target Type:	Chemical
Background:	Sensitive determination of NAD and NADH by colorimetric (565nm) method.

Target Details

	Procedure: 15 min. Pyridine nucleotides play an important role in metabolism and, thus, there is continual interest in monitoring their concentration levels. Quantitative determination of NAD + /NADH has applications in research pertaining to energy transformation and redox state of cells or tissue. Simple, direct and automation-ready procedures for measuring NAD + /NADH concentration are very desirable. This NAD + /NADH assay kit is based on a lactate dehydrogenase cycling reaction, in which the formed NADH reduces a formazan (MTT) reagent. The intensity of the reduced product color, measured at 565 nm, is proportionate to the NAD + /NADH concentration in the sample. This assay is highly specific for NAD + /NADH and with minimal interference (< 1%) by NADP + /NADPH. Our assay is a convenient method to measure NAD, NADH and their ratio.

Application Details

Application Notes:	Direct Assays: NAD + /NADH concentrations and ratios in cell or tissue extracts.
Comment:	1. At these concentrations, the standard curves for NAD and NADH are identical. Since NADH in solution is unstable, we provide only NAD as the standard. 2. This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended. 3. The following substances interfere and should be avoided in sample preparation. EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).
Protocol:	Add 80 µL Working Reagent per well quickly. Tap plate to mix briefly and thoroughly. Read optical density (OD0) for time zero at 565 nm (520-600nm) and OD15 after a 15-min incubation at room temperature. Calculation: Subtract OD0 from OD15 for the standard and sample wells. Use the OD values to determine sample NAD/NADH concentration from the standard curve. Note: If the sample OD values are higher than the OD value for the 10 µMstandard, dilute sample in distilled water and repeat this assay. Multiply the results by the dilution factor.
Reagent Preparation:	For each well of reaction, prepare Working Reagent by mixing 60 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 14 µL Lactate and 14 µL MTT. Fresh reconstitution is recommended.
Sample Preparation:	For tissues weigh ~20 mg tissue for each sample, wash with cold PBS. For cell samples, wash cells with cold PBS and pellet ~10 5 cells for each sample. Homogenize samples (either tissue or cells) in a1.5 mL Eppindorf tube with either 100 µL NAD extraction buffer for NAD

Application Details

	determination or 100 µL NADH extraction buffer for NADH determination. Heat extracts at 60°C for 5 min and then add 20 µL Assay Buffer and 100 µL of the opposite extraction buffer to neutralize the extracts. Briefly vortex and spin the samples down at 14,000 rpm for 5 min. Use supernatant for NAD/NADH assays. Determination of both NAD and NADH concentrations requires extractions from two separate samples
Restrictions:	For Research Use only

Handling

Storage:	4 °C

Publications

Product cited in:

Bai, Cantó, Oudart, Brunyánszki, Cen, Thomas, Yamamoto, Huber, Kiss, Houtkooper, Schoonjans, Schreiber, Sauve, Menissier-de Murcia, Auwerx: "PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation." in: **Cell metabolism**, Vol. 13, Issue 4, pp. 461-8, (2011) (PubMed).

Greenall, Lei, Swan, James, Wang, Peters, Wipat, Wilkinson, Lydall: "A genome wide analysis of the response to uncapped telomeres in budding yeast reveals a novel role for the NAD+ biosynthetic gene BNA2 in chromosome end protection." in: **Genome biology**, Vol. 9, Issue 10, pp. R146, (2010) (PubMed).

Lee, Cho, Jantaratnotai, Wang, McGeer, McGeer: "Depletion of GSH in glial cells induces neurotoxicity: relevance to aging and degenerative neurological diseases." in: **FASEB journal : official publication of the Federation of American Societies for Experimental Biology**, Vol. 24, Issue 7, pp. 2533-45, (2010) (PubMed).

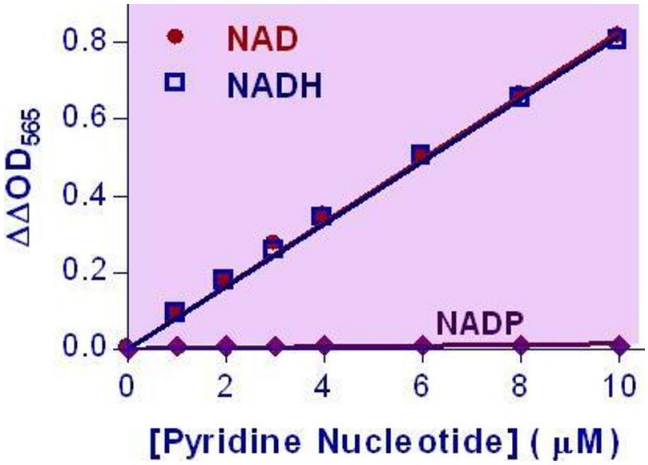
Koo, Gong, Kim, Kim, Lee: "Improvement of coenzyme Q(10) production by increasing the NADH/NAD(+) ratio in Agrobacterium tumefaciens." in: **Bioscience, biotechnology, and biochemistry**, Vol. 74, Issue 4, pp. 895-8, (2010) (PubMed).

Thornburg, Nelson, Clem, Lane, Arumugam, Simmons, Eaton, Telang, Chesney: "Targeting aspartate aminotransferase in breast cancer." in: **Breast cancer research : BCR**, Vol. 10, Issue 5 , pp. R84, (2009) (PubMed).

Publications

There are more publications referencing this product on: Product page

Images



Biochemical Assay

Image 1.