Tribioscience

NADH/NAD Quantification Colorimetric Assay (Catalog: TBS2029, 100 Assays, Store at -20 ℃)

DESCRIPTION

Nicotinamide adenine dinucleotide (NAD) is an enzymatic cofactor involved in many redox reactions. NAD functions as an electron carrier, cycling between the oxidized (NAD) and reduced (NADH) forms. In addition to its role in redox reactions, NAD plays critical roles in ADP (adenosine diphosphate)-ribosylation reactions and as a substrate for sirtuins.

The Tribio™ NADH/NAD Quantification Colorimetric Assay is based on a dehydrogenase coupled reaction convert WST-8 to WST-8 formazan, which can be measured at OD 460 nm. The generated signal is proportional to the content of NADH. This assay increases the detection sensitivity by purification of NAD and NADH from the cell lysate. The kit provides an easiest and accurate approach to measure NADH from a variety of samples.

APPLICATIONS

Cell and biological tissues.

KIT CONTENTS FOR 100 TESTS:

Name	Size (100 tests)
Assay Buffer	10 mL
Enzyme mix	50 µL
Enzyme substrate mix	50 µL
NADH Detection Probe (5X)	1 mL
NADH Standard stock (2 mM)	400 µL
NAD/NADH Extraction Buffer	30 mL

Storage conditions: Store the Reagent at –20°C protected from light. Shelf life: 6 months.

PROCEDURES

1. Preparation of NADH Standards as Table.

Std#	NADH standard (µL)	Assay Buffer (µL)	NADHConcentration (µM)	Total NADH (nmol/well)
1	50 (2 mM stock)	150	500	25
2	200 (Std1)	200	250	12.5
3	200 (Std2)	200	125	6.25
4	200 (Std3)	200	62.5	3.125
5	200 (Std4)	200	31.25	1.563
6	200 (Std5)	200	15.625	0.781
7	200 (Std6)	200	7.813	0.391
8	Blank (0)	400	0	0.0

2.Sample preparation (NAD/NADH Extraction)

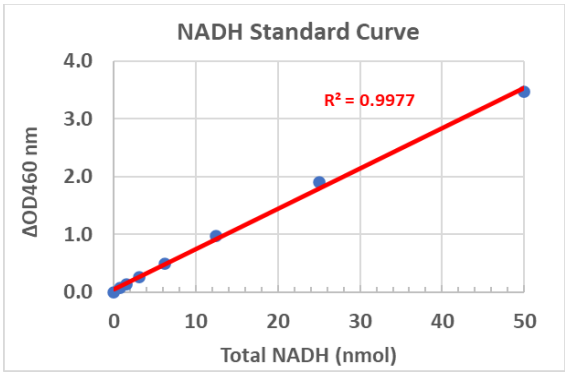
1) For cell samples: Wash the cells with 400 µL cold PBS.
2) Pellet 2 X 10^5 cells: for each assay in a micro-centrifuge tube (2000 rpm for 5 min.).
3) Aspirate the wash solution from the tube.
4) Add 300 µl of NADH/NAD Extraction Buffer by freeze / thaw two cycles (20 min. on dry-ice, then 10 min. at RT), or by homogenization.
5) Vortex the extraction for 30 sec.
   (For tissues, weigh ~20 mg tissue & wash with cold PBS. Homogenize in 300 µl of NADH/NAD Extraction Buffer in a micro-centrifuge tube).
6) Centrifuge at 14000 rpm for 5 min.
7) Transfer the extracted NADH/NAD supernatant into 2 labeled tubs. One is for Total NADH (NADH and NAD) detection, and another tube for NADH detection.
8) To detect total NADH, transfer 50 µl of extracted samples into labeled 96-well plate. To detect NADH, NAD needs to be decomposed before the reaction. To decompose NAD, Heat the tube to 60 °C for 30 min. in a water bath or a heating block. Under this condition, all NAD will decompose, while NADH will still be intact. Cool samples on ice. Quick spin the samples to remove precipitates if precipitation occurs. Transfer 50 µl of NAD decomposed samples into labeled 96-well plate.

3. Preparation of NAD+/NADH reaction mix, please adjust the volume as you need.
Mixing the below reagent for 100 tests: total volume is 5 mL.

- 3900 µL Assay Buffer.
- 50 µL Enzyme mix.
- 50 µL Substrate mix.
- 1000 µL Detection Probe.

4. Load samples:
Add 50 µL of NADH Standard and the test sample NAD^+, NADH preparation to each well. (Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).

5. Add 50 µL of reaction mix to each well containing the standards and the test samples.
6. Incubate at room temperature for about 20 ~ 60 minutes, with gentle shaking and protected from light.
7. Measure $OD_{460\ nm}$.
8. Calculation:
Typical NADH standard curve as below:



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Apply the sample OD reading to the standard curve to get the total NADH and the NADH amount in the sample wells.

NAD^+ = Total NADH-NADH

NAD^+/NADH ratio = NAD^+ Concentration / NADH concentration

RELATIVE PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
CCK-8 Cell Viability Assay (TBS2022)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
ADP/ATP Ratio Assay Kit (Bioluminescent (BS2015)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Caspase-3 Colorimetric Assay kit (TBS2030)
Caspase-3 Fluorometric Assay kit (TBS2035)

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