NAD/NADH Assay (NAD)

Cat. No. 8368
100 Tests in 96-well plate

Introduction

Nicotinamide adenine dinucleotide (NAD), which acts as a soluble electron carrier between proteins, is an important enzymatic cofactor involved in many redox reactions. NAD functions in modulating cellular redox status by controlling signaling and transcriptional events, making it and related enzymes drug targets for various metabolic disorders. This colorimetric assay is based on lactate dehydrogenase catalyzed reduction of NAD, in which the formed NADH can reduce tetrazolium salt INT to an INT-formazan product. The intensity of the INT-formazan product, which exhibits maximum absorbance at 490 nm, is proportional to the amount of NADt (NAD and NADH) or NADH in the sample. This assay is specific for NAD and NADH, and it does not detect NADP or NADPH.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8368a	1	Assay buffer	10 mL	4°C
8368b	1	Extraction buffer	50 mL	4°C
8368c	1	Lactate solution	1.0 mL	-20°C
8368d	1	NAD standard	0.3 mL	-20°C
8368e	1	INT	0.2 mL	-20°C
8368f	1	Enzyme mix	0.2 mL	-20°C

Product Use

This assay measures NADt (NAD and NADH) and NADH concentrations and NAD/NADH ratio in cell and tissue extracts. It is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from NAD/NADH Assay of NAD solutions with concentrations ranging from 0.5 to 10 µM show a linear relationship between OD_{490nm} and NAD concentration (Figure 1).

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of NAD standard

1. Add 1 µL of NAD standard (8368d) to 499 µL of assay buffer (8368a) to make a 0.5 mL solution of 20 µM NAD.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8368a) into each tube and label them #1 through #7.
3. Add 25 µL of the 20 µM NAD into tube #1 and mix well to get the 10 µM NAD standard.
4. Transfer 25 µL of the 10 µM NAD standard from tube #1 to tube #2 and mix well to get the 5 µM NAD standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the NAD standards. Do not add any NAD to tube #7, which will serve as the blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each NAD standard by aliquoting 10 µL/well of each NAD standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	10 µM	5 µM	2.5 µM	1.25 µM	0.625 µM	0.3125 µM	Blank
B	10 µM	5 µM	2.5 µM	1.25 µM	0.625 µM	0.3125 µM	Blank

B. Preparation of test samples

1. For cell samples, wash cells with cold PBS and pellet 2 X 10^5 cells for each sample. For tissue samples, use around 20 mg tissue for each sample and wash them with cold PBS. Cell samples can be homogenized or freeze/thawed for 2 cycles (20 minutes on dry-ice, then 10 minutes at room temperature) in 400 µl of the extraction buffer (8368b). Tissue samples can be homogenized in 400 µl of the extraction buffer (8368b). Centrifuge the samples at 13,000 ×g for 10 minutes. The extracted supernatant will be used as the test sample.
2. To detect total NADt (NAD and NADH), transfer 10 µl of extracted test samples into labeled 96-well plate in duplicates. To detect NADH, NAD needs to be decomposed before the reaction. To decompose NAD, aliquot 100 µl the extracted test samples into tubes. Heat it at 60°C for 30 min. Under the condition, all NAD will be decomposed, while NADH will still be intact. Cool samples on ice. Quick spin the samples. Transfer 10 µl of NAD decomposed samples into labeled 96-well plate in duplicates.
3. Test samples should be serial diluted to ensure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well plate.

Recommendation:

Cell and tissues lysate may contain enzymes that consume NAD or NADH. Samples should be deproteinized with a 10k Da MW spin filter (Millipore UFC501008) or 0.5M metaphosphoric acid (Sigma 239275) to remove these enzymes and kept at -80°C for storage.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare the working reagent by mixing 76 µL assay buffer (8368a), 10 µL lactate solution (8368c), 2 µL INT (8368e), and 2 µL enzyme mix (8368f).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing NAD standard, test

samples, and the blank. Incubate for 20 minutes at room temperature protected from light.
3. Read the absorbance at 490 nm with an ELISA plate reader.

D. Calculations

1. Subtract the OD_{490nm} value of the blank from the OD_{490nm} values obtained with all other standard and samples to get ΔOD_{490nm} value.
2. Based on the calibrated ΔOD_{490nm} of the NAD standard, make a standard curve by plotting ΔOD_{490nm} as a function of NAD concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.
3. Because the equation of the trend line of the standard curve is $y = Ax + B$, calculate the NADH or NADt concentration of test samples as follows:

$$[\text{NADH}] \text{ or } [\text{NADt}] = \frac{\Delta\ OD_{490nm} - B}{A}$$

4. The ratio of NAD+/NADH in the sample may be calculated by the following equation:

$$\text{NAD/NADH} = \frac{\text{NADt - NADH}}{\text{NADH}}$$

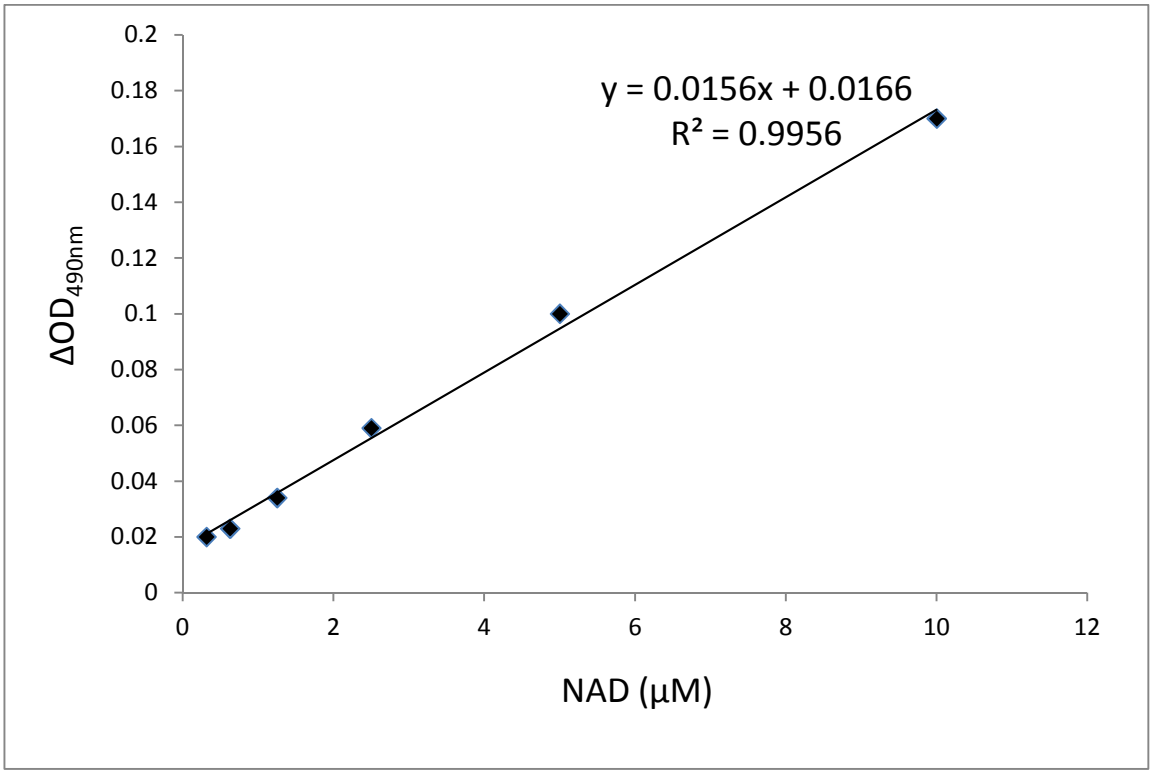

Figure1. A typical NAD standard curve measured by ScienCell™ NAD/NADH Assay kit