NAD-Malate Dehydrogenase (NAD-MDH) Assay Kit

Catalog No.: abx295106

Size: 96 tests

Detection Range: 1.06 U/L – 46.7 U/L

Sensitivity: 1.06 U/L

Storage: Store all components at -20°C. Store the Substrate, Chromogenic Reagent, and Standards in the dark.

Application: For detection and quantification of NAD-Malate Dehydrogenase activity in serum, plasma, tissue homogenates, and cell lysates.

Introduction

Abbexa's NAD-Malate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating NAD-Malate Dehydrogenase activity. NAD-MDH catalyzes the reaction of NAD and Malic acid, forming Oxaloacetic acid and NADH. NADH is oxidized while WST-8 is converted to orange-colored formazan, which has an absorbance maximum at 450 nm. The intensity of the color change over time is proportional to the NAD-Malate Dehydrogenase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 60 ml
3. Buffer Solution A: 20 ml
4. Buffer Solution B: 10 ml
5. Substrate: 1 vial
6. Chromogenic Reagent: 6 ml
7. Standard: 2 vials
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum/Plasma:** Samples can be tested directly. Assay immediately, or store at -80°C for up to 1 month.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue into a centrifuge tube, then add 180 µl Extraction Solution. Homogenize at 4°C with a Dounce homogenizer, then centrifuge at 10,000 × g for 15 minutes. Collect the supernatant and store on ice for immediate assay. The protein content of the supernatant should be determined separately **(abx097193)**.
- **Cell Lysates:** Harvest 1 × 10^6 cells and wash with PBS (0.01 M, pH 7.4). Homogenize in 200 µl Extraction Solution by ultrasonication at 4°C, then centrifuge at 10,000 × g for 10 minutes. Collect the supernatant and store on ice for immediate assay. The protein content of the supernatant should be determined separately **(abx097193)**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10 % Rat kidney tissue homogenate	1
10 % Rat spleen tissue homogenate	1
10 % Rat liver tissue homogenate	1
10 % Rat heart tissue homogenate	1
10 % Mouse liver tissue homogenate	1
10 % Mouse lung tissue homogenate	1
Mouse serum/plasma	1
Rat serum/plasma	1
Human serum	1
Dog serum	1
HT29 cell lysate	1
Molt-4 cell lysate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Dissolve the vial of substrate in 5 ml of double distilled water. Prepared solution can be stored at -20°C for up to 5 days. Avoid repeated freeze-thaw cycles.
- **Reaction Working Solution:** *Prepare sufficient working solution for 160 µl per assay well*. Example: Mix 100 µl of Buffer Solution A, 40 µl of Buffer Solution B, and 20 µl of Substrate Working Solution. Prepare working solution fresh before assay and use within 1 hour.
- **500 µmol/L Standard Solution:** Dissolve a vial of Standard in 1.6 ml of Extraction Solution. Prepared solution can be stored at -20°C for up to 5 days. Avoid repeated freeze-thaw cycles.
- **Standard Dilutions:** Prepare Standard dilutions by serial dilution of 500 µmol/L Standard Solution, as summarized in the following table:

Standard Dilution (µmol/L)	0	100	150	200	300	350	400	500
500 µmol/L Standard Solution (µl)	0	20	30	40	60	70	80	100
Extraction Solution (µl)	100	80	70	60	40	30	20	0

For the blank, or 0 mg/ml standard, use pure Extraction Solution. The volume of each standard will be 100 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Avoid foaming when adding Reaction Working Solution.

B. Assay Procedure

1. Assign microplate wells for each standard or sample.
2. Add 20 µl of diluted standards to standard wells.
3. Add 20 µl of sample to sample wells.
4. Add 160 µl of Reaction Working Solution to each well. Avoid foaming.
5. Add 40 µl of Chromogenic Reagent to each well and mix fully.
6. Incubate at room temperature for 2 minutes in the dark, then measure the OD of each well with a microplate reader at 450 nm. Record the OD values as A_1.
7. Incubate at 37°C for 10 minutes in the dark, then measure the OD of each well with a microplate reader at 450 nm. Record the OD values as A_2. *Note: Standard wells will show no change in OD between A_1 and A_2.*

C. Calculation of Results

Plot the standard curve, **using the A_2 OD value** of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of NAD-Malate Dehydrogenase in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

One unit of NAD-Malate Dehydrogenase activity is defined as the amount of NAD-MDH in 1 L of sample that produces 1 µmol of NADH per minute at 37°C.

$$\text{NAD-MDH Activity (U/L)} = \frac{\Delta \boldsymbol{A_{450}} - \boldsymbol{b}}{\boldsymbol{a} \times \boldsymbol{T}} \times \boldsymbol{f}$$

2. Tissue Homogenate and Cell Lysate samples:

One unit of NAD-Malate Dehydrogenase activity is defined as the amount of NAD-MDH in 1 g of sample protein content that produces 1 µmol of NADH per minute at 37°C.

$$\text{NAD-MDH Activity (U/g protein)} = \frac{\Delta \boldsymbol{A_{450}} - \boldsymbol{b}}{\boldsymbol{a} \times \boldsymbol{T}} \times \frac{\boldsymbol{f}}{\boldsymbol{C_{protein}}}$$

where:

ΔA_{450}	OD change in sample wells (A_2-A_1)
T	Reaction incubation time (10 minutes)
$C_{protein}$	Protein content of sample (g protein/L)
f	Sample dilution factor
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)

D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.