ELISA PRODUCT INFORMATION & MANUAL

Malate Dehydrogenase Activity Assay Kit (Fluorometric)

NBP2-59750

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Table of Contents

1.	Overview	1
2.	Materials Supplied and Storage	2
3.	Materials Required, Not Supplied	3
4.	General guidelines, precautions, and troubleshooting	4
5.	Reagent Preparation	5
6.	Standard Preparation	7
7.	Sample Preparation	9
8.	Assay Procedure	11
9.	Data Analysis	13
10.	Typical Data	14
11.	Notes	16

1. Overview

The Malate Dehydrogenase Activity Assay Kit (NBP2-59750) is a quick and easy method for monitoring malate dehydrogenase (MDH) activity in wide variety of samples. In this assay, MDH converts malate into intermediate products, which will further react with the Probe forming a fluorophore (Ex/Em=535/587nm). The kit is simple, sensitive and high-throughput adaptable and can detect as low as 40 µU of MDH activity.

Prepare all samples/standards as directed.

Prepare the NADH standard curve.

Prepare enough reaction mix for 50 µl per well.

Measure Fluorescence (Ex/Em= 535/587 nm) immediately in kinetic mode for 10-60 min. at 37°C.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
MDH Assay Buffer	25 ml	-20°C	-20°C/4°C
MDH Substrate	1 vial	-20°C	-20°C
MDH Probe	450 µl	-20°C	-20°C
MDH Developer	1 vial	-20°C	-20°C
NADH Standard	1 vial	-20°C	-20°C
MDH Positive Control	1 vial	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Dounce homogenizer (if using tissue).
- 96-well opaque white plate with flat bottom.
- Multi-well spectrophotometer capable of measuring absorbance (Ex/Em= 535/587 nm) in kinetic mode.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening. – adapt if necessary.

5.1 MDH Assay Buffer

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at 4°C or -20°C.

5.2 MDH Substrate

1. Reconstitute with 220 µl Assay Buffer.
2. Keep on ice while in use.

5.3 MDH Probe

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Keep away from light.

5.4 MDH Developer

1. Reconstitute with 220 µl Assay buffer.
2. Aliquot and store at -20°C. Keep on ice while in use.

5.5 NADH Standard

1. Reconstitute with 400 µl dH2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution.
2. Aliquot and store at -20°C. Keep on ice while in use.

5.6 MDH Positive control

1. Reconstitute with 400 µl Assay Buffer to the Positive Control and mix thoroughly.
2. Aliquot and store at -20°C Keep on ice while in use.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 50 µM (50 pmol/µL) NADH Standard by adding 4 µL of the reconstituted NADH standard into 96 µL of distilled water.
2. Using 50 µM (50 pmol/µL) NADH Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	50 p/mol/µl Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount of NADH standard in well (pmol/well)
1	0	100	50	0
2	4	96	50	100
3	8	92	50	200
4	12	88	50	300
5	16	84	50	400
6	20	80	50	500

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay. Include if samples can be frozen: If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.

7.1 Tissue samples

1. Rapidly homogenize tissue (~10 mg) with 100 µl ice cold MDH Assay Buffer and keep on ice for 10 minutes.
2. Centrifuge at 10,000 x *g* for 5 minutes at 4 °C and transfer the supernatant to a fresh tube.
3. Add 2-50 µl sample per well in a white plate and adjust the volume to 50 µl with MDH Assay Buffer.

7.2 Cell samples (adherent or suspension)

1. Rapidly homogenize cells (~1 x 10^6) with 100 µl ice cold MDH Assay Buffer, and keep on ice for 10 minutes.
2. Centrifuge at 10,000 x *g* for 5 minutes at 4 °C and transfer the supernatant to a fresh tube.
3. Add 2-50 µl sample per well in a white plate and adjust the volume to 50 µl with MDH Assay Buffer.

7.3 For mitochondrial MDH activity.

1. Isolate the mitochondria from fresh tissue or cells using Mitochondria isolation kit for tissue and cultured cells.

2. Add 2-50 µL of isolated mitochondria per well in a 96-well white plate, adjust the volume to 50 µL with MDH Assay buffer.

3. For the MDH positive control: dilute the MDH positive control to 50X of the stock solution by adding 4 µL of the reconstituted MDH positive control (see section 5.6) into 196 µL of MDH Assay Buffer.

4. Take 2-20 µL of MDH Positive control into desired well(s) and adjust the final volume to 50 µL with MDH Assay Buffer.

∆**Note:** For samples exhibiting significant background, prepare parallel sample well(s) as sample background controls.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.
-

Δ Note: If you suspect your samples contain substance that can generate background, set up Sample Background Controls to correct for background noise

8.1 Reaction wells set up:

1. Set up all standard, control and sample wells as previously described.

8.2 Reaction mix:

1. Prepare 50 µL of Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
MDH Assay Buffer	44	46
MDH Developer	2	2
MDH Probe	2	2
MDH Substrate	2	---

2. Add 50 µL of Reaction Mix into each standard, control and sample wells.
3. Add 50 µL of Background Reaction Mix into the background control sample wells.

8.3 Measurement

Measure Fluorescence (Ex/Em= 535/587 nm) immediately in kinetic mode for 10-60 minutes at 37°C.

∆**Note:** Incubation time depends on the malate dehydrogenase activity in samples. We recommend measuring the fluorescence in kinetic mode, and choosing two time points (t1 and t2) in the linear range to calculate the malate dehydrogenase activity of the samples. The NADH Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time).

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of NADH.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. Calculate the malate dehydrogenase activity of the test sample: ΔRFU = RFU2 – RFU1. Apply the ΔRFU to the NADH Standard Curve to get B pmol of NADH generated during the reaction time (Δt = t2 - t1).

$$\text{Sample Malate Dehydrogenase Activity} = \frac{B}{(\Delta t \times V)} \times D$$

Where:

B = amount of NADH in the sample well calculated from standard curve in (pmol).

Δt =Reaction time (min).

V = sample volume added in the reaction well (µL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

Δ**Note:** One unit of malate dehydrogenase is the amount of enzyme that generates 1.0 µmol of NADH per min at pH 9.5 at 37°C.

10. Typical Data

Data provided **for demonstration purposes only**.

Figure 1: NADH standard curve.

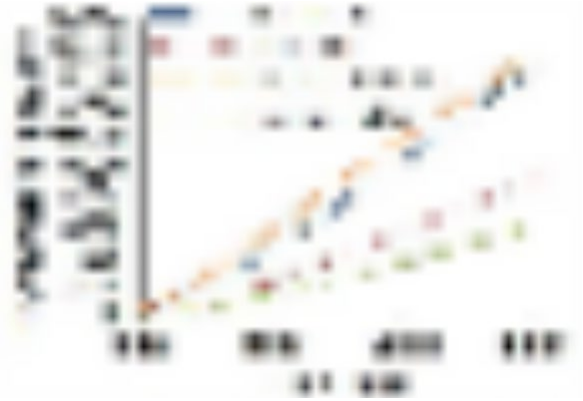

Figure 2: Malate Dehydrogenase activities in rat muscle extract (0.011 µg protein), Jurkat cell lysate (0.026 µg protein), Saccharomyces mitochondria (0.05 µg) and MDH positive control

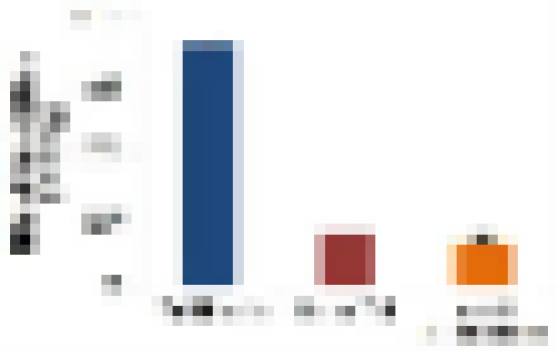

Figure 3: Specific MDH activities in rat muscle extract, Jurkat cell lysate and yeast mitochondria. Assays were performed following the kit protocol.

11. Notes