

MDH Activity Colorimetric Assay Kit

Cat. No. Kit-0544 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	MDH Activity Assay Kit (Colorimetric) is used for measuring malate dehydrogenase activity using colorimetric methods.
Description	Malate Dehydrogenase (MDH) (EC 1.1.1.37) is an important enzyme which reversibly converts L-malate into oxaloacetate in the presence of NAD. In eukaryotic cells, malate dehydrogenase has 2 isoforms: MDH1 and MDH2. MDH1 is cytosolic & participates in the malate- aspartate shuttle, which transports malate into mitochondria for utilization in ATP generation whereas MDH2 is a mitochondrial enzyme and part of the citric acid cycle. MDH activity is increased in some neurodegenerative diseases such as Alzheimer's disease, and abnormal MDH activity in serum can serve as a diagnostic tool for severe liver damage (e.g. Hepatocellular carcinoma). In Malate Dehydrogenase Activity Assay kit, MDH reacts with malate to form an intermediate. The generated intermediate reacts with MDH Developer to form a colored product with strong absorbance at 450 nm. The assay is simple, sensitive and can detect less than 0.5 mU of MDH activity in various sample types.
Applications	Measurement of malate dehydrogenase activity in various tissues/cells Analysis of citric acid cycle and malate-aspartate shuttle
Usage	For research use only (RUO)
Storage	Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the experiment.

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Kit Components	MDH Assay Buffer 20 ml MDH Substrate (Lyophilized) 1 vial MDH Enzyme Mix (Lyophilized) 1 vial MDH Developer (Lyophilized) 1 vial NADH Standard (Lyophilized) 1 vial MDH Positive Control (Lyophilized) 1 vial
Materials Required but Not Supplied	96-well clear plate with flat bottom Multi-well spectrophotometer
Detection method	Colorimetric
Compatible Sample Types	Cell culture, Tissue samples
Preparation	MDH Assay Buffer: Warm to room temperature before use. Store at either 4°C or -20°C. MDH Substrate: Reconstitute with 220 µAssay Buffer. Store at -20°C. Keep on ice while in use. Use within two months. MDH Enzyme Mix: Reconstitute with 220 µAssay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months. MDH Developer: Reconstitute with 1.05 ml dH₂O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months. NADH Standard: Reconstitute with 400 µdH₂O to generate 1.25 mM (1.25 nmol/l) NADH Standard solution. Aliquot & store at -20°C. Keep on ice while in use. Use within two months. MDH Positive Control: Reconstitute with 400 µAssay Buffer and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

Assay Protocol

1. Sample Preparation: Rapidly homogenize tissue (10 mg) or cells (1×10^6) with 100 μ l cold MDH Assay Buffer. Keep on ice for 10 min. Centrifuge at $10,000 \times g$ for 5 min. at 4°C and collect the supernatant. Add 1-50 μ l sample per well & adjust the volume to 50 μ l with MDH Assay Buffer. To check mitochondrial MDH activity, isolate mitochondria from fresh tissues or cells. Add 1-50 μ l of isolated mitochondria per well & adjust the volume to 50 μ l with MDH Assay Buffer. Add 1-10 μ l of MDH Positive Control into desired well(s) & adjust the volume to 50 μ l with MDH Assay Buffer.

Notes:

For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

For samples having background, prepare parallel sample well(s) as sample background control(s).

Small molecules in some tissue samples such as heart may interfere with the assay. To remove small molecules, we recommend using ammonium sulfate method to precipitate the enzymes.

Transfer tissue homogenate (50 μ l) to a clean centrifuge tube & add 2 volumes of saturated ammonium sulfate (4.1 M). Keep on ice for 20 min. & centrifuge at $10,000 \times g$ for 5 min. at 4°C .

Discard the supernatant and suspend the pellet in MDH Assay Buffer to the original volume.

2. NADH Standard Curve: Add 0, 2, 4, 6, 8 and 10 μ l of 1.25 mM NADH Standard into a series of wells in 96-well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust the volume to 50 μ l/well with MDH Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 μ l Mix containing:

Reaction Mix *Background Control Mix

MDH Assay Buffer 36 μ l 38 μ l

MDH Enzyme Mix 2 μ l 2 μ l

MDH Developer 10 μ l 10 μ l

MDH Substrate 2 μ l ---

Add 50 µl of the Reaction Mix to each well containing Standards, Positive Control and test samples.

* For samples having high background, add 50 µl of Background Control mix to sample background control well(s). Mix well.

4. Measurement: Measure absorbance at 450 nm in kinetic mode for 10-30 min. at 37°C.

Note: Incubation time depends on the malate dehydrogenase activity in the samples. We recommend measuring the OD in a kinetic mode, and choosing two time points (T1 & 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 incubation time).

Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. If sample background control reading is significant then subtract sample background reading from sample reading. Calculate the MDH activity of the test samples: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by malate dehydrogenase during the reaction time ($\Delta T = T_2 - T_1$).

Sample Malate Dehydrogenase Activity = $B / (\Delta T \times V) \times D$ = nmol/min/ml = mU/ml

Where:

B is NADH amount from the Standard Curve (nmol)

ΔT is reaction time (min)

V is sample volume added into the reaction well (ml)

D is sample Dilution Factor

Specific activity of Malate Dehydrogenase can be expressed as mU/mg of protein.

Unit Definition: 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.

Analysis

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