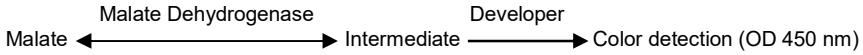
Malate Dehydrogenase Activity Colorimetric Assay Kit (#BN00878)

(Catalog # BN00878; 100 assays; Store at -20°C)

I. Introduction:

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 Assay Genie's 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.



II. Application:

- Measurement of malate dehydrogenase activity in various tissues/cells
- Analysis of citric acid cycle and malate-asparate shuttle

III. Sample Type:

- Animal tissues such as liver, heart, muscle, etc.
- Cell culture: Adherent or suspension cells
- Mitochondria

IV. Kit Contents:

Components	BN00878	Cap Code	Part Number
MDH Assay Buffer	20 ml	WM	BN00878-1
MDH Substrate (Lyophilized)	1 vial	Blue	BN00878-2
MDH Enzyme Mix (Lyophilized)	1 vial	Green	BN00878-3
MDH Developer (Lyophilized)	1 vial	Red	BN00878-4
NADH Standard (Lyophilized)	1 vial	Yellow	BN00878-5
MDH Positive Control (Lyophilized)	1 vial	Orange	BN00878-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **MDH Assay Buffer:** Warm to room temperature before use. Store at either 4°C or -20°C.
- **MDH Substrate:** Reconstitute with 220 µl Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.
- **MDH Enzyme Mix:** Reconstitute with 220 µl Assay 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 dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Aliquot & store at -20°C. Keep on ice while in use. Use within two months.
- **MDH Positive Control:** Reconstitute with 400 µl Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VIII. Malate Dehydrogenase Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold MDH Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x g for 5 min. at 4°C and collect the supernatant. Add 1-50 µl sample per well & adjust the volume to 50 µl with MDH Assay Buffer. To check mitochondrial MDH activity, isolate mitochondria from fresh tissues or cells using Assay Genie Mitochondrial Isolation Kit for Tissue & Cultured Cells (# BN00546). Add 1-50 µl of isolated mitochondria per well & adjust the volume to 50 µl with MDH Assay Buffer. Add 1-10 µl of MDH Positive Control into desired well(s) & adjust the volume to 50 µl with MDH Assay Buffer.

   Notes:

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

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

   c. 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 µl) 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 x 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 µl 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 µl/well with MDH Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	*Background Control Mix
MDH Assay Buffer	36 µl	38 µl
MDH Enzyme Mix	2 µl	2 µl
MDH Developer	10 µl	10 µl
MDH Substrate	2 µl	----

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

* For samples having high background, and 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 (T_1 & T_2) 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).

5. **Calculation:** 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/(ΔT X V) x D = nmol/min/ml = mU/ml

Where: **B** is NADH amount from the Standard Curve (nmol)
Δ**T** 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.

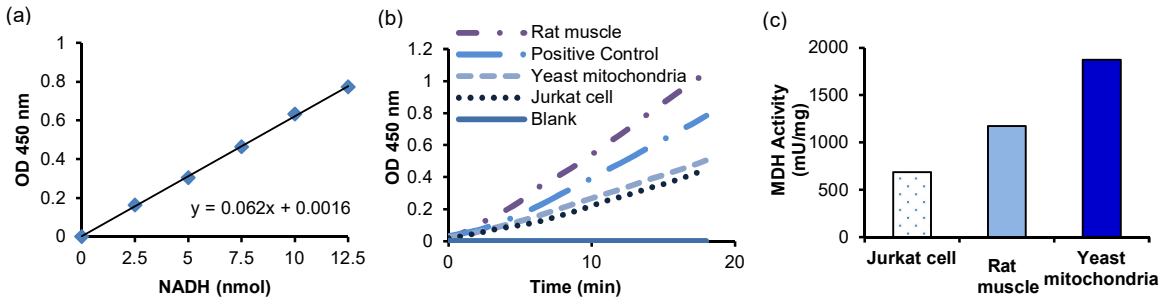


Figure: NADH Standard Curve (a). Malate Dehydrogenase activity in rat muscle extract (0.8 µg), Jurkat cell lysate (0.6 µg), yeast mitochondria (1.2 µg) & MDH Positive Control (b). Referenced MDH Activity in Jurkat cell lysate, rat muscle extract and yeast mitochondrial lysate (c). Assays were performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.