Product Information

Malate Dehydrogenase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2206-100 100 assays	Cap Color	Part Number
MDH Assay Buffer	20 ml	WM	K2206-C-1
MDH Substrate (Lyophilized)	1 vial	Blue	K2206-C-2
MDH Enzyme Mix (Lyophilized)	1 vial	Green	K2206-C-3
MDH Developer (Lyophilized)	1 vial	Red	K2206-C-4
NADH Standard (Lyophilized)	1 vial	Yellow	K2206-C-5
MDH Positive Control (Lyophilized)	1 vial	Orange	K2206-C-6

II. Introduction:

Malate Dehydrogenase (MDH) is an enzyme that reversibly catalyzes the oxidation of L-malate to oxaloacetate in the presence of NAD^+. MDH has 2 isoforms: MDH1 and MDH2. MDH1 exists in cytosolic and participates in the malate-aspartate shuttle, which transports malate into mitochondria for ATP production. However, MDH2 is present in mitochondria and is involved in the citric acid cycle. In severe liver damage such as hepatocellular carcinoma, MDH activity in serum is abnormal, which can be used as a diagnostic tool. In addition, MDH activity is increased in some neurodegenerative diseases such as Alzheimer's disease.

The Malate Dehydrogenase Activity Colorimetric Assay Kit provides a highly sensitive, simple, fast and convenient way for detection of MDH activity in various biological samples based on colorimetric method. In the assay, MDH reacts with malate to form an intermediate, which reacts with MDH Developer to yield a colored product with strong absorbance at 450 nm. The kit can detect less than 0.5 mU MDH activity in various sample types.

III. Application:

Measurement of malate dehydrogenase activity in various tissues/cells.

Analysis of citric acid cycle and malate-asparate shuttle.

IV. Sample Type:

Animal tissues such as liver, heart, muscle, etc.

Cell culture: Adherent or suspension cells.

Mitochondria.

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. 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 (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).

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: $\Delta OD = A2 - A1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by malate dehydrogenase during the reaction time ($\Delta T = T2 - T1$).

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℃.

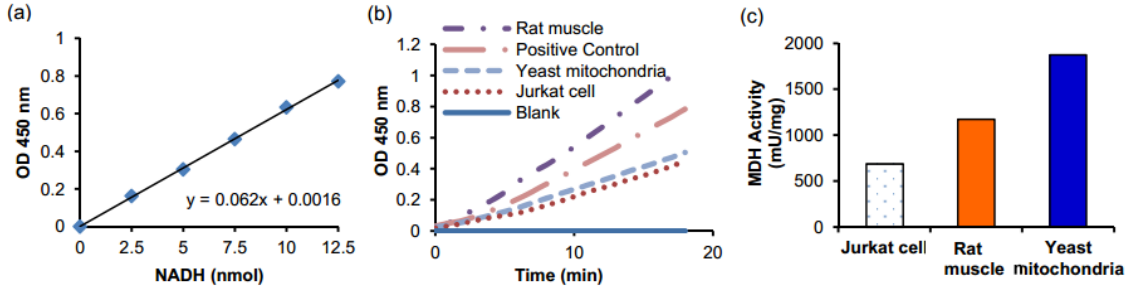

Figure: NADH Standard Curve (a). Malate Dehydrogenase activity in rat muscle extract (0.8 µg), Jurkat cell lysate (0.6 µg), yeast mitochondria (1.2 µg) & MDH Positive Control (b). Referenced MDH Activity in Jurkat cell lysate, rat muscle extract and yeast mitochondrial lysate (c). Assays were performed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

Frequently Asked Questions

1. Does it rely on an immunoprecipitation step to isolate MDH from tissues? Also, is it possible to use purified protein for this assay? If so, is there a recommended working range?

No, there is no immunopreceipitation done in this assay. Purified MDH enzyme can be used . The readings need to be within the linear range of the std curve. How much sample is used needs to be optimized to achieve this. This kit can detect from 0.1 - 2.5 mU of enzyme activity.

2. Can mitochondrial MDH activity be measured?

Mitochondria isolated from the tissue/cell sample of interest and then solubilized using the assay buffer can be used to measure mitochondrial MDH specifically.

3. Does the MDH positive control consist of MDH1 protein, MDH2 protein, or both?

The positive control is from mitochondria (MDH2).

4. Which isoform of MDH is meassure by this kit?

It depends on which sub-cellular fraction is used for the assay. Total cell lysate will measure both MDH 1 and 2., cytosolic fraction will be from MDH1 and mitochondria will have MDH2.

5. Can this assay be normalized with respect to protein concentration?

Yes, a detergent-compatible BCA assay can be used for protein quantitation to normalize sample amount.

6. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, it is strongly recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

7. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples since this is an enzyme activity assay. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

8. Can cells lysed in RIPA buffer be used with this kit?

RIPA buffer typically contains SDS which might affect the function of the enzymes in the kit. Hence we do not recommend using RIPA bufefr samples. The assay buffer in the kit also provides optimum conditions for the enzymes to work at their best.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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