ab183305

Malate Dehydrogenase Activity Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Malate Dehydrogenase in various samples.

View kit datasheet: www.abcam.com/ab183305
(use www.abcam.cn/ab183305 for China, or www.abcam.co.jp/ab183305 for Japan)

This product is for research use only and is not intended for diagnostic use.

Version 2b, Last Updated 23 January 2024

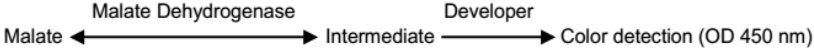
Table of Contents

1. Overview

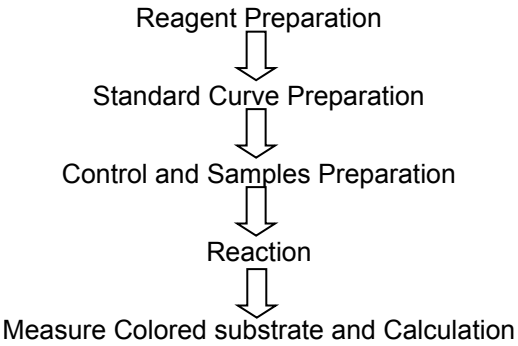
Malate Dehydrogenase (MDH) (EC 1.1.1.37) is an important enzyme which reversibly converts L-malate into oxaloacetate in the presence of NAD. In eukaryotic cells, malate dehydrogenase has 2 isoforms: MDH1 and MDH2. MDH1 is cytosolic and participates in the malateaspartate shuttle, which transports malate 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 Abcam's Malate Dehydrogenase Activity Assay kit (Colorimetric) (ab183305), 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.

Figure 1: Assay Procedure



2. Protocol Summary



3. Kits Components

Item	Quantity
MDH Assay Buffer	25 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

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Distilled water (dH_2O)
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option

6. Reagents Preparation

1. **MDH Assay Buffer:**
   Ready to use as supplied. Aliquot and store at +4°C or -20°C. Warm to room temperature before use.

2. **MDH Substrate:**
   Reconstitute with 220 µL MDH Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.

3. **MDH Enzyme Mix:**
   Reconstitute with 220 µL MDH Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.

4. **MDH Developer:**
   Reconstitute with 1.05 mL dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.

5. **NADH Standard**
   Reconstitute with 400 µL dH_2O to generate 1.25 mM (1.25 nmol/µL) NADH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

6. **MDH Positive Control**

   Reconstitute with 400 µL MDH Assay Buffer and dissolve completely. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.

7. Assay Protocol

1. **Sample Preparation**

   Cells (starting material: 10^6 cells) or tissues (starting material 10mg)

   Homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µL ice cold MDH Assay Buffer on ice. Keep on ice for 10 minutes. Centrifuge at 10,000 X g for 5 minutes at +4°C. Collect the supernatant. Add 1-50 µL samples per well. Adjust the final volume to 50 µL with MDH Assay Buffer. To check mitochondrial MDH activity, isolate mitochondria from fresh tissues or cells using ab110170, ab110168 or another user defined kit. Add 1-50 µL isolated mitochondria per well and adjust the volume to 50 µL with MDH Assay Buffer. Add 1-10 µL of MDH Positive Control into the desired well(s) and adjust the final volume to 50 µL with MDH Assay Buffer.

   NOTE:

   - *Small molecules in some tissue samples such as heart may interfere with the assay. To remove small molecules,*

we recommend using ammonium sulphate method to precipitate the enzymes. Transfer tissue homogenate (50 µL) to a clean centrifuge tube and add 2 volumes of saturated ammonium sulphate (4.1 M). Keep on ice for 20 minutes and centrifuge at 10,000 x g for 5 minutes at 4°C. Discard the supernatant and suspend the pellet in MDH Assay Buffer to the original volume.

- *For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.*
- *For samples having high background, prepare parallel sample well(s) as the background control.*

2. Standard Curve Preparation:

a) 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.

b) Adjust the volume to 50 µL/well with MDH Assay Buffer.

3. Reaction Mix:

Prepare enough Reaction Mix 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 the 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 (OD 450 nm) immediately in kinetic mode for 10-30 minutes at 37ºC.

NOTE:

- *Incubation time depends on the MDH Activity in the samples. We recommend measuring the OD in a kinetic mode, and choosing two time points (T_1 and T_2) in the linear range to calculate the MDH Activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).*

8. Data Analysis

Calculations:

a) Subtract 0 Standard reading from all Standard readings. Plot the NADH Standard Curve. Correct sample reading by subtracting the value derived from the background control 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 MDH during the reaction time ($\Delta T = T_2 - T_1$).

Sample MDH Activity = B/(ΔT X V) x Dilution Factor = nmol/min/µL = mU/µL or U mL

Where:
B is the NADH amount from Standard Curve (nmol)
V is the sample volume added into the reaction well (µL)
Δ**T** is the reaction time (minutes)

Unit Definition: One unit of MDH is the amount of enzyme that will generate 1.0 µmol of NADH per minute at pH 9.5 at 37ºC.

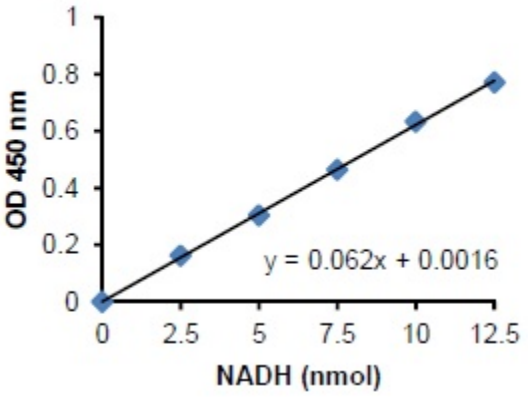


Figure 2. NADH Standard Curve.

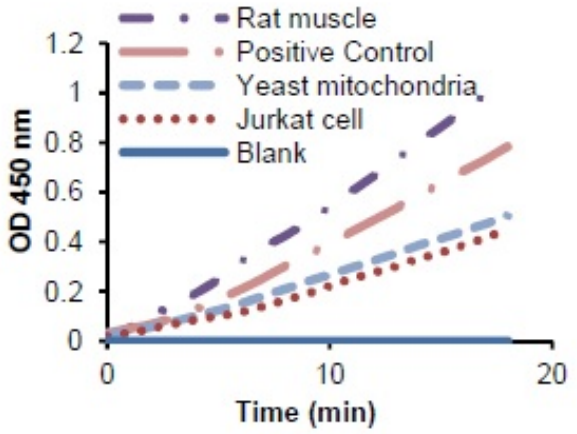


Figure 3. MDH activity in rat muscle extract (0.8 µg), Jurkat cell lysate (0.6 µg), yeast mitochondria (1.2 µg) and MDH positive control. Assays were performed following the kit protocol.

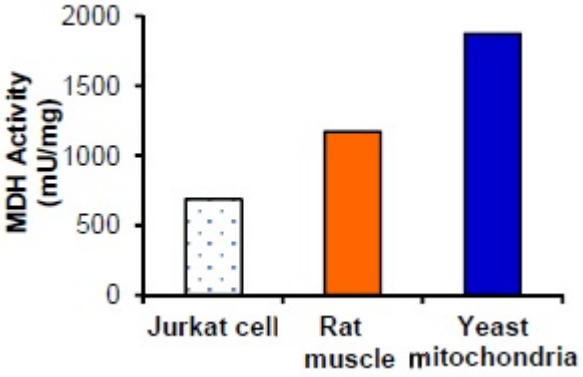


Figure 4. Referenced MDH Activity in Jurkat cell lysate, rat muscle extract and yeast mitochondrial lysate.

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)