

# **ab200009 – Malate Dehydrogenase 1 (MDH1) Activity Assay**

## Instructions for Use

For the rapid, sensitive and accurate measurement of Malate Dehydrogenase 1 (MDH1) Activity in Human, mouse, and rat samples.

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

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## 1. BACKGROUND

Abcam's Malate Dehydrogenase 1 (MDH1) Activity Assay kit is designed for the sensitive and accurate measurement of MDH1 activity in Human, mouse, and rat samples.

Malate Dehydrogenase 1 is the cytoplasmic isoform of the enzyme responsible for catalyzing the reversible oxidation of malate to oxaloacetate. The enzyme's activity requires the NAD<sup>+</sup>/NADH cofactor and participates primarily in the malate-aspartate shuttle.

The enzyme activity is determined by following the production of NADH in the following MDH1 catalyzed reaction:



The generation of NADH is coupled to the 1:1 reduction of a reporter dye to yield a colored (yellow) reaction product whose concentration can be monitored by measuring the increase in absorbance at 450nm (Dye molar extinction coefficient: 37000M<sup>-1</sup> cm<sup>-1</sup>). In each well, only native MDH1 is immunocaptured from the sample; this removes all other enzymes, including MDH2 from the activity measurement.

## 2. ASSAY SUMMARY

Prepare samples as instructed.  
Determine the protein concentration of extracts.



Equilibrate all reagents to room temperature.



Dilute sample to desired protein concentration in 1X Incubation Buffer.  
Add 100  $\mu$ L sample to each well used. Incubate 2 hours at room temperature.



Aspirate and wash each well three times.

Freshly prepare 1X Activity Solution then add 200  $\mu$ L to each well. Pop bubbles and immediately record the color development at 450 nm for 10 to 45 minutes. *Alternatively, measure the endpoint at a user-determined time.*

### 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

### 4. STORAGE AND STABILITY

**Store kit at +2-8°C immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9&10.

### 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
20X Buffer	20 mL	+2-8°C
Extraction Buffer	15 mL	+2-8°C
10X Blocking Buffer	8 mL	+2-8°C
Base Buffer	24 mL	+2-8°C
100X NAD <sup>+</sup> (Lyophilized)	1 vial	+2-8°C
100X Sodium Malate (Lyophilized)	1 vial	+2-8°C
100X Reagent Dye (Lyophilized)	1 vial	+2-8°C
100X Coupler (Lyophilized)	1 vial	+2-8°C
Pre-coated Microplate (12 x 8 well strips)	96 Wells	+2-8°C

### **6. MATERIALS REQUIRED, NOT SUPPLIED**

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

- Microplate reader capable of measuring absorbance at 450nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi and single channel pipettes.
- Tubes for sample dilution.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors) and/or phosphatase inhibitors.

### **7. LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

### 8. TECHNICAL HINTS

- A visualized color change right after adding the reaction buffer indicates fast reaction. Samples, therefore, should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 10).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating sample dilution series, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

## 9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

### 9.1 1X Wash Buffer

Prepare Wash Buffer by adding 10 mL 20X Buffer to 190 mL nanopure water. Mix gently and thoroughly.

### 9.2 1X Incubation Buffer

Prepare 1X Incubation Buffer by adding 5 mL 10X Blocking Buffer to 45 mL 1X Wash Buffer. Mix gently and thoroughly.

### 9.3 100X NAD<sup>+</sup>

Resuspend the lyophilized NAD<sup>+</sup> by adding 250  $\mu$ L nanopure water. Mix gently and thoroughly. After resuspension any unused 100X NAD<sup>+</sup> should be aliquoted and stored at -20°C. Avoid multiple freeze/thaws.

### 9.4 100X Sodium Malate

Resuspend the lyophilized Sodium Malate by adding 250  $\mu$ L nanopure water. Mix gently and thoroughly. After resuspension any unused 100X Sodium Malate should be aliquoted and stored at -20°C. Avoid multiple freeze/thaws.

### 9.5 100X Reagent Dye

Resuspend the lyophilized Reagent Dye by adding 250  $\mu$ L nanopure water. Mix gently and thoroughly. After resuspension any unused 100X Reagent Dye should be aliquoted and stored at -20°C. Avoid multiple freeze/thaws.

### 9.6 100X Coupler

Resuspend the lyophilized Coupler by adding 250  $\mu$ L nanopure water. Mix gently and thoroughly. After resuspension any unused 100X Coupler should be aliquoted and stored at -20°C. Avoid multiple freeze/thaws.

### 9.7 Reaction Buffer

Prepare the Reaction Buffer immediately prior to use. Prepare 2 mL Reaction Buffer for each 8 well strip used. Use

## ASSAY PREPARATION

the table below for instructions on how to prepare the necessary volume of Reaction Buffer:

Base Buffer (μL)	100X NAD+ (μL)	100X Sodium Malate (μL)	100X Reagent Dye (μL)	100X Coupler (μL)	Total (μL)
1,920	20	20	20	20	2,000
3,840	40	40	40	40	4,000
5,760	60	60	60	60	6,000
7,680	80	80	80	80	8,000
9,600	100	100	100	100	10,000
11,520	120	120	120	120	12,000
13,440	140	140	140	140	14,000
15,360	160	160	160	160	16,000
17,280	180	180	180	180	18,000
19,200	200	200	200	200	20,000
21,120	220	220	220	220	22,000
23,040	240	240	240	240	24,000

## 10. SAMPLE PREPARATION

### TYPICAL SAMPLE DYNAMIC RANGE -

Typical working ranges	
Sample Type	Range (µg/mL)
HeLa Extract	15 – 1,000
Human Heart Homogenate (HHH)	15 – 1,000
Human Liver Homogenate (HLH)	15 – 1,000
Human Skeletal Muscle Homogenate (HSkH)	15 – 1,000
Mouse Liver Homogenate (MLH)	15 – 1,000
Rat Heart Homogenate (RHH)	15 – 1,000
Rat Liver Homogenate (RLH)	15 – 1,000
HepG2 Cell Lysate	15 – 1,000

### 10.1 Preparation of extracts from cell pellets

- 10.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.
- 10.1.2 Rinse cells twice with PBS.
- 10.1.3 Solubilize cell pellet at  $2 \times 10^7$ /mL in Extraction Buffer.
- 10.1.4 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

## 10.2 Preparation of extracts from tissue homogenates

- 10.2.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
- 10.2.2 Homogenize 100 to 200 mg of wet tissue in 500  $\mu$ L – 1 mL of Extraction Buffer. For lower amounts of tissue adjust volumes accordingly.
- 10.2.3 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

## 11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

## 12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

12.1 Prepare all reagents as directed in section 9 & 10.

12.2 Samples should be diluted within the working range of the assay in 1X Incubation Buffer.

12.3 Add 100  $\mu$ L sample into each well.

12.4 Incubate for 2 hours at room temperature.

12.5 Wash three times with 1X Wash Buffer.

12.6 Add 200  $\mu$ L of Reaction Buffer.

*Note: Change tips between different Reaction Buffers to prevent contamination of samples. Quickly remove any visible bubbles.*

12.7 Immediately record the yellow color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	450 nm
Time:	up to 45 min.
Interval:	20 sec.
Shaking:	Shake before and between readings

*Alternative*– In place of a kinetic reading, at a *user defined*, time record the endpoint OD data at 450 nm.

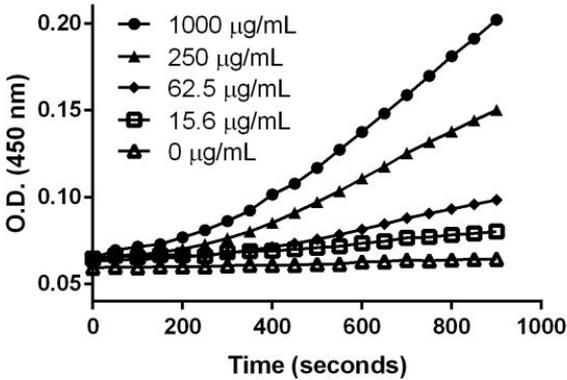
## 13. CALCULATIONS

MDH1 activity in each well is proportional to the increase in absorbance at 450 nm within each well. MDH1 specific activity is calculated by subtracting the OD value of the sample well from the OD value of the background well. The activity is expressed as the change in absorbance per minute per amount of sample loaded into the well. Examine the linear rate of increase in absorbance at 450 nm over time. Most microplate software is capable of performing this function.

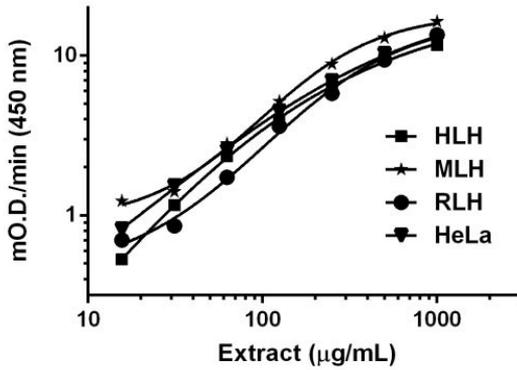
The starting concentration of malate in the assay is 5 mM and NAD<sup>+</sup> is 4 mM. Malate oxidation and production of NADH by MDH1 in each well is 1:1 proportional with dye reduction and an increase in absorbance at 450 nm (dye  $\epsilon_{450\text{nm}} = 37 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

## 14. TYPICAL DATA

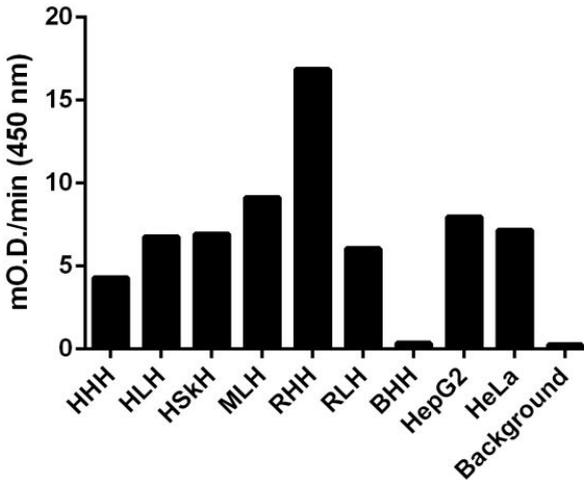
An example is shown below where the rate/slope is calculated from the linear portion of the data.



**Figure 1.** Raw data from HeLa cell lysate. Once the rate/slope of each lane is extracted from the linear range of the time point data, it is expressed as rate (mOD/min) per microgram of cell lysate added per well. A lag time of a few minutes is possible before the mOD/min reaches the linear range.



**Figure 2:** Representative background-subtracted kinetic measurements from serially diluted Hela cell lysates and tissue homogenates HLH, MLH, RLH.



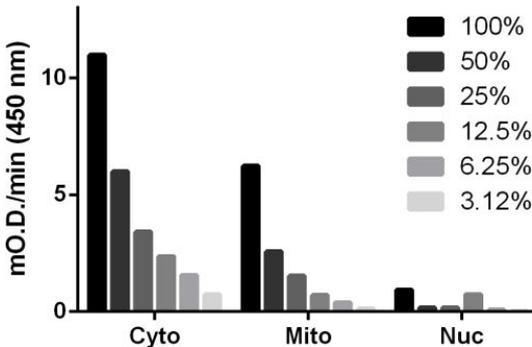
**Figure 3.** The assay was used to determine the MDH1 activity in a series of normal cell lysates and tissue homogenates loaded at 250 µg/mL.

## ASSAY REPRODUCIBILITY

	Intra- Assay	Inter- Assay
n=	4	4
%CV	3.4	8.1

## 15. ASSAY SPECIFICITY

This assay uses an antibody specific to MDH1 to isolate the enzyme from the solubilized cell or tissue extracts. In this way it is possible to measure only the activity from MDH1 without any competing signal from MDH2. This specificity was demonstrated by assaying the MDH1 activity of HeLa cells fractionated into mitochondrial, cytoplasmic, and nuclear fractions. Although the fractionation does not produce purified cellular components, the relative enrichment of MDH1 in the cytoplasmic fraction demonstrates the specificity.



**Figure 4.** MDH1 activity in serially titrated HeLa cell fractions (Cyto = Cytoplasmic, Mito = Mitochondrial and Nuc = Nuclear) demonstrates the assay's specificity to MDH1.

## 16. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Assay not working	Assay buffer at wrong temperature	Assay buffer needs to be at room temperature
	Component missed in the Reaction Buffer	Prepare fresh buffers and follow protocol exactly
	Insufficient amount of enzyme	Try loading samples at a higher concentration
Unexpected results	Plate read at incorrect wavelength	Use appropriate reader and filter settings described in datasheet
	Sample readings are outside linear range	Adjust concentrations of samples to be within the linear range of the assay
	Sample prepared in an unsuitable extraction reagent	Use the extraction buffer included in the kit to prepare lysates
Inconsistent readings	Sample has undergone too many freeze/ thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
	Kit components not fully thawed prior to beginning the assay	Wait for components to thaw completely and gently mix prior use
	Inaccurate Pipetting	Check pipettes

## RESOURCES

Inconsistent readings	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

17. NOTES

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