



Malate Dehydrogenase Microplate Assay Kit User Manual

Catalog # CAK1266

(Version 1.1A)

Detection and Quantification of Malate Dehydrogenase (MDH)
Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture
media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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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).

Malate Dehydrogenase Microplate Assay Kit provides a simple and direct procedure for measuring malate dehydrogenase activity in a variety of samples. In this kit, malate dehydrogenase catalyzed oxidation of malate in which the formed NADH reduces a formazan reagent. The rate of decrease in the absorbency at 450 nm, resulting from the oxidation of NADH, is a measure of MDH activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
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Note:

Substrate: add 1 ml Reaction Buffer to dissolve before use.

Dye Reagent A: add 1 ml distilled water to dissolve before use, mix, store at 4°C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

Positive Control: add 1 ml assay buffer to dissolve before use, then add 0.1 ml into 0.8 ml assay buffer, mix. Aliquot & store at -80 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	80 μ l	80 μ l	--	--	80 μ l
Sample	10 μ l	--	--	--	--
Standard	--	--	100 μ l	--	--
Distilled water	--	10 μ l	--	100 μ l	--
Positive Control	--	--	--	--	10 μ l
Substrate	10 μ l	10 μ l	--	--	10 μ l
Dye Reagent A	90 μ l	90 μ l	90 μ l	90 μ l	90 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
Mix, keep in dark for 5 minutes at room temperature, record absorbance measured at 450 nm.					

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of MDK activity is defined as the enzyme converts one 1 μmol of NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{MDK (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 0.8 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{MDK (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times W / V_{\text{Assay}}) / T \\ &= 0.8 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{MDK (U/10}^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 0.8 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned} \text{MDK (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &\quad / T \\ &= 0.8 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

C_{Standard} : the standard concentration, $400 \mu\text{mol/L} = 0.4 \mu\text{mol/ml}$;

V_{Standard} : the volume of standard, $100 \mu\text{l} = 0.1 \text{ ml}$;

V_{Sample} : the volume of sample, 0.01 ml ;

C_{Protein} : the protein concentration, mg/ml ;

W : the weight of sample, g ;

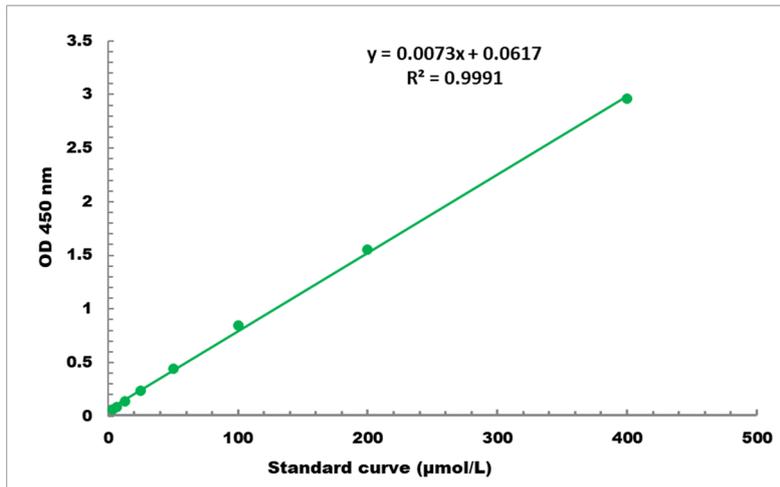
N : the quantity of cell or bacteria, $N \times 10^4$;

V_{Assay} : the volume of Assay buffer, 1 ml ;

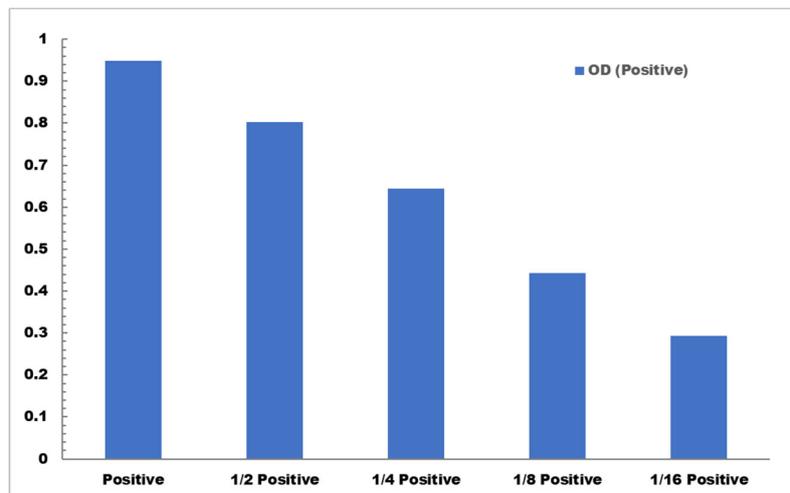
T : the reaction time, 5 minutes .

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES