CheKine™ Micro Mitochondrial Malate Dehydrogenase (mMDH) Activity Assay Kit

Cat #: KTB1280 Size: 48 T/96 T

	Micro Mitochondrial Malate Dehydrogenase (mMDH) Activity Assay Kit		
REF	**Cat #:** KTB1280	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Malate Dehydrogenase (MDH, EC 1.1.1.37) is widely present in animals, plants, microorganisms and cultured cells. MDH in mitochondria is one of the key enzymes in the TCA cycle and catalyzes the formation of oxaloacetate from malate; on the contrary, MDH in the cytoplasm catalyzes oxaloacetate. Malic acid is formed. Oxaloacetate is an important intermediate product that connects many important metabolic pathways. Therefore, MDH plays an important role in various physiological activities of cells, including mitochondrial energy metabolism, malate-aspartate shuttle system, reactive oxygen species metabolism, and disease resistance. According to different coenzyme specificities, MDH is divided into NAD-dependent MDH and NADP-dependent MDH. Bacteria usually only contain NAD-MDH. In eukaryotic cells, NAD-MDH is distributed in the cytoplasm and mitochondria. CheKine™ Micro Mitochondrial Malate Dehydrogenase (mMDH) Activity Assay Kit provides a simple, convenient and rapid mMDH activity detection method, which is suitable for the detection of animal tissues, plant tissues, cells, bacteria, serum, plasma and other samples. The principle is that mMDH catalyzes the reduction of oxaloacetate by NADH to malate, resulting in a decrease in light absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	0.75 mL	1.5 mL	-20℃, protected from light
Reagent Ⅲ	10 mL	20 mL	4℃
Reagent Ⅳ	1	1	-20℃, protected from light

Materials Required but Not Supplied

• Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm

- 96-well UV microplate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice maker, refrigerated centrifuge, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

ReagentⅢ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Solution: Prepare before use. For 48 T, add 9.5 mL of ReagentⅢand 0.25 mL of deionized water to ReagentⅣ. For 96 T, add 19 mL of ReagentⅢ and 0.5 mL of deionized water to ReagentⅣ, mix well; The remaining reagents should be stored at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Plasma and serum: Direct detection.
2. Extraction of cytoplasmic protein and mitochondrial protein from cells, bacteria and tissue

(1) Weigh 0.1 g tissue or collect 5×10^6 cells and bacteria, add 1 mL Extraction Buffer and 10 μL Reagent Ⅱ, homogenize on ice. Centrifuge at 600 g for 5 min at 4℃. Collect the supernatant to a new centrifuge tube and discard the pellet.

(2) Centrifuge the supernatant again at 11,000 g for 10 min at 4℃, and obtain the supernatant and precipitate respectively.

(3) (Optional) The supernatant collected in step (2) is cytoplasmic extract, which can be used to directly determine mMDH leaking from mitochondria.

(4) Add 200 μL Reagent Ⅰ and 2 μL Reagent Ⅱ to the precipitate collected in step (2), resuspend the precipitate sufficiently, and use it to detect the activity of mMDH in the next step.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the samples are placed for a long time, different operation habits or other reasons, which resulted in a large amount of mMDH in the supernatant, then the supernatant must be tested.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Incubate Working Solution for 10 min at 37°C (mammal) or 25°C (other species).
3. Add 5 μL of sample, 195 μL of Working Solution in a 96-well UV plate or microquartz cuvette. After mixing quickly, record the absorbance values of 20 s and 1 min 20 s at 340 nm with a microplate reader, mark as A_1 and A_2, and calculate $\Delta A=A_1-A_2$.

Note: If the sample absorbance value ΔA is greater than 0.5, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

1. Calculation of mMDH activity in serum (plasma)

Unit definition: one enzyme activity unit defines as 1 nmol NADH consumed by each mL of serum (plasma) per min in the reaction system.

mMDH activity (U/mL)=[ΔA×V_{total} ÷(ε×d)×10^9]÷V_{sample}÷T**=12,861.74×ΔA**

2. Calculated by fresh weight of samples

Unit definition: one enzyme activity unit defines as 1 nmol NADH consumed by 1 g tissue per min in the reaction system.

$mMDH_{Supernatant}$ activity (U/g fresh weight)=[$\Delta A_{Supernatant}$×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Extraction}$×W)÷T**=12,990.35×$\Delta A_{Supernatant}$÷W**

$mMDH_{Pellet}$ activity (U/g fresh weight)=[ΔA_{Pellet}×V_{Total} ÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×W)÷T**=2,598.07×ΔA_{Pellet}÷W**

Total mMDH activity (U/g fresh weight)=$mMDH_{Supernatant}$ activity+$mMDH_{Pellet}$ activity**=12,990.35×$\Delta A_{Supernatant}$÷W+2,598.07×ΔA_{Pellet}÷W**

3. Calculated by cells or bacteria density

Unit definition: one enzyme activity unit defines as 1 nmol NADH consumed by 10^4 cells or bacteria per min in the reaction system.

mMDH activity (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×500)÷T**=5.2×ΔA**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficient, 6.22×10^3 mol/L/cm; d: 0.5 cm; V_{Sample}: sample volume added, 0.005 mL; T: reaction time, 1 min; $\Delta A_{Supernatant}$: OD value of supernatant; $V_{Extraction}$: sample extract volume, 1.01 mL; W: sample weight, g; ΔA_{Pellet}: OD value of pellet; $V_{Total\ Sample}$: the volume of adding Reagent Ⅰ and Ⅱ, 0.202 mL; 500: total number of cells or bacteria, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase (SDH) Activity Assay Kit
KTB1240	CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.