CheKine™ Micro Methylcitrate Synthase (MCS) Activity Assay Kit

Cat #: KTB1027 Size: 48 T/96 T

	Micro Methylcitrate Synthase (MCS) Activity Assay Kit		
REF	**Cat #:** KTB1027	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Methylcitrate synthase (MCS) exists widely in the mitochondrial matrix of animals, plants, microorganisms and cultured cells, and participates in the regulation of tricarboxylic acid cycle together with citrate synthase (CS). CheKine™ Micro Methylcitrate Synthase (MCS) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues,Cells, Serum or other Liquid samples. In this kit, MCS catalyzes propionyl CoA and oxaloacetic acid to produce methyl citric acid, which further hydrolyzes to methyl citric acid, which promotes the conversion of colorless DTNB to yellow TNB with characteristic absorbance at 412 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4℃
Reagent Ⅱ	10 mL	20 mL	4℃
Reagent Ⅲ	0.75 mL	1.5 mL	4℃
Reagent Ⅳ	15 mL	30 mL	4℃
Reagent Ⅴ	1	1	4℃
Reagent Ⅵ	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water, absolute ethanol
- Homogenizer or mortar (for tissue samples)

Abbkine, Inc • USA • ***www.abbkine.com***
Not for further distribution without written consent.Copyright © Abbkine, Inc.

Reagent Preparation

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 4℃.

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

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

Reagent V： Prepared before use. Add 0.5 mL absolute ethanol and 11 mL Reagent IV for 48 T, 1 mL absolute ethanol and 22 mL Reagent IV for 96 T to fully dissolve. The unused reagents are sub-packaged and stored at -20℃ for 6 months, and repeated freezing and thawing is prohibited.

Reagent VI： Prepared before use. Add 1.5 mL deionized water for 48 T and 3 mL deionized water for 96 T to fully dissolve. The unused reagents are sub-packaged and stored at -20℃ for 6 months, and repeated freezing and thawing is prohibited.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Separation of cytoplasmic and mitochondrial proteins from tissues: Weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Reagent I and 10 μL Reagent III, homogenize on ice. Centrifuge at 600 g for 5 min at 4℃. Abandon precipitation, and transfer the supernatant to another centrifugal tube;Centrifuge at 11,000 g for 5 min at 4℃. The supernatant is to remove the cytoplasmic protein of mitochondria, which can be used to determine the MCS leakage from mitochondria (optional). The precipitation is mitochondria, adding 200 μL Reagent II and 2 μL Reagent III, ultrasonic fragmentation (ice bath, power 20% or 200 W, ultrasound 3 s, interval 10 s, repetition 30 times) for MCS activity determination.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent V at 37℃ (mammals) or 25℃ (other species) for 10 min.
3. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well (μL)
Sample	20
Reagent VI	220
Reagent V	10

4. Mix thoroughly, detect the absorbance at 412 nm as A_1 immediately and A_2 after 10 min. Finally calculate $\Delta A=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity appropriately. If A is greater than 1.5 or ΔA is greater than 1, the sample can be appropriately diluted with Reagent I or Reagent II, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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 UV plates calculation formula as below

Calculation of the MCS activity

Abbkine, Inc • USA • www.abbkine.com

Not for further distribution without written consent.Copyright © Abbkine, Inc.

(1) Calculated by fresh weight of samples

Unit definition: The production of 1 nmol TNB per min in g tissue is defined as a unit of enzyme activity.

$MCS_{Supernatant}$ (U/g weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Extraction}\times V_{Sample})\div T$**=92.83×$\Delta A_{Supernatant}$÷W**

$MCS_{Precipitate}$ (U/g weight)=$[\Delta A_{Precipitate}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Total\ Sample}\times V_{Sample})\div T$**=18.57×$\Delta A_{Precipitate}$÷W**

Total MCS (U/g weight)=$MCS_{Supernatant}+MCS_{Precipitate}$**=92.83×$\Delta A_{Supernatant}$÷W+18.57×$\Delta A_{Precipitate}$÷W**

(2) Calculated by cell of samples

Unit definition: The production of 1 nmol TNB per min in 10^4 cells is defined as a unit of enzyme activity.

$MCS_{Supernatant}$ (U/10^4 cell)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(n\div V_{Extraction}\times V_{Sample})\div T$**=92.83×$\Delta A_{Supernatant}$÷n**

$MCS_{Precipitate}$ (U/10^4 cell)=$[\Delta A_{Precipitate}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(n\div V_{Total\ Sample}\times V_{Sample})\div T$**=18.57×$\Delta A_{Precipitate}$÷n**

Total MCS (U/10^4 cell)=$MCS_{Supernatant}+MCS_{Precipitate}$**=92.83×$\Delta A_{Supernatant}$÷n+18.57×$\Delta A_{Precipitate}$÷n**

$\Delta A_{Supernatant}$: OD value of supernatant; $\Delta A_{Precipitate}$: OD value of precipitate; V_{Total}: total reaction volume, 2.5×10^{-4} L; ε: TNB molar extinction coefficient, 1.36×10^4 L/mol /cm; d: 0.5 cm; V_{Sample}: sample volume added, 0.02 mL; $V_{Extraction}$: the volume of adding Reagent Ⅰ and Ⅲ, 1.01 mL; $V_{Total\ Sample}$: the volume of adding Reagent Ⅱ and Ⅲ, 0.202 mL; T: reaction time, 20 min; W: sample weight, g; n: total number of cells, in tens of thousands.

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.

Precautions

1. During the determination, the sample and all reagents are placed on the ice to avoid denaturation and inactivation.
2. Try to extract MCS from fresh samples to ensure the activity of the enzyme.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

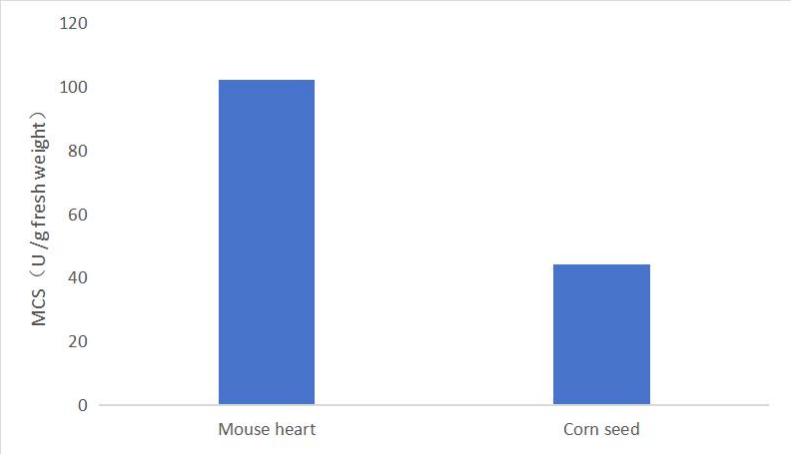

Figure 1. Determination of MCS activity in mouse heart and corn seed by this kit.

Recommended Products

Catalog No.	Product Name
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit

Disclaimer

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

Not for further distribution without written consent.Copyright © Abbkine, Inc.