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CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit

Cat #: KTB1023 Size: 48 T/48 S 96 T/96 S

	Micro Citrate Synthase (CS) Activity Assay Kit		
REF	**Cat #:** KTB1023	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Citrate Synthase (CS) widely exists in the mitochondrial matrix of animals, plants, microorganisms, and cultured cells. It is the first rate-limiting enzyme and one of the main regulatory sites of Kreb's cycle. CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit provides a simple method for detecting CS activity in animal and plant tissues, cells. CS activity is determined depending on that CS can catalyze acetyl CoA and oxamic acid to produce citroyl co-enzyme A, further hydrolysis to produce citric acid; this reaction makes colorless DTNB transform into yellow TNB, TNB has a characteristic absorbing value at 412 nm. The activity of citrate synthase can be obtained by calculating the increase rate of the light absorption at 412 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	12 mL	24 mL	4℃
Reagent Ⅱ	0.75 mL	1.5 mL	-20℃, protected from light
Reagent Ⅲ	15 mL	30 mL	4℃
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅵ	Powder×1 vial	Powder×1 vial	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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
- Refrigerated centrifuge, water bath, ice maker, 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. Store at -20℃, protected from light.

Note: Reagent Ⅱ is toxic, so it is recommended to experiment in a fume hood.

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

Working Reagent: Before use, resolve ReagentⅣ and ReagentⅤ with 13.2 mL ReagentⅢ for 48 T; resolve ReagentⅣ and ReagentⅤ with 26.4 mL ReagentⅢ for 96 T. The remaining reagents should be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Working ReagentⅥ: Before use, add 0.6 mL deionized water to fully dissolve for 48 T; add 1.2 mL deionized water to fully dissolve for 96 T. The remaining reagents should be stored at -20℃ and protected from light for 1 month 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. Processed samples must be assayed immediately. There may be enzyme activity in both supernatant and Precipitate, so it is recommended to detect both parts for more accurate results.

Extraction of cytoplasmic protein and mitochondrial protein from cells and tissue:

1. Weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Extraction Buffer and 10 μL ReagentⅡ, 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 determine CS leaking from mitochondria.
4. Add 200 μL ReagentⅠ and 2 μL ReagentⅡ to the precipitate collected in step 2, mix well, and use it to detect the activity of CS in the next step.

Note: The samples extracted by this kit can also be used for the determination of KTB1270, KTB1290, KTB1280, KTB1240, KTB1230, KTB1250.

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. Preheated Working Reagent for 5 min in 37℃ (mammal) or 25℃ (other species) water bath.
3. Add 10 μL of sample, 220 μL of Working Reagent, and then 10 μL of Working ReagentⅥ in a 96-well plate or microglass cuvette. After mixing quickly, record the absorbance values of 20 s and 2 min 20 s at 412 nm with a microplate reader, mark as A_1 and A_2, and 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 ΔA is larger than 0.3 the sample can be appropriately diluted with corresponding Extraction Buffer, 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 plates calculation formula

1. Calculation of CS activity in tissue of the sample (according to the weight of the sample):

Unit definition: an enzyme activity unit defines as 1 g tissue catalyzes the oxidation of 1 nmol TNB per min in the reaction system at 37℃ (mammal) or 25℃ (other species).

$CS_{Supernatant}$ (U/g weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Extraction}\times V_{Sample})\div T$**=1,782.35×$\Delta A_{Supernatant}$÷W**

$CS_{Precipitate}$ (U/g weight)=$[\Delta A_{Precipitate}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\div V_{Total\ Sample}\times V_{Sample})\div T$**=356.47×$\Delta A_{Precipitate}$÷W**

Total CS (U/g weight)=$CS_{Supernatant}+CS_{Precipitate}$**=1,782.35×$\Delta A_{Supernatant}$÷W+356.47×$\Delta A_{Precipitate}$÷W**

2. Calculation of CS activity in cells (according to the number of cells):

Unit definition: an enzyme activity unit defines as 10,000 cells catalyze the oxidation of 1 nmol TNB per min in the reaction system at 37℃ (mammal) or 25℃ (other species).

$CS_{Supernatant}$ (U/10^4 cell)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(N\div V_{Extraction}\times V_{Sample})\div T$**=1,782.35×$\Delta A_{Supernatant}$÷N**

$CS_{Precipitate}$ (U/10^4 cell)=$[\Delta A_{Precipitate}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(N\div V_{Total\ Sample}\times V_{Sample})\div T$**=356.47×$\Delta A_{Precipitate}$÷N**

Total CS (U/10^4 cell)=$CS_{Supernatant}+CS_{Precipitate}$**=1,782.35×$\Delta A_{Supernatant}$÷N+356.47×$\Delta A_{Precipitate}$÷N**

Where: $\Delta A_{Supernatant}$: OD value of supernatant; $\Delta A_{Precipitate}$: OD value of precipitate; V_{Total}: total reaction volume, 2.4×10^{-4} L; ε: TNB molar extinction coefficient, 1.36×10^4 L/mol /cm; d: 0.5 cm; V_{Sample}: sample volume added, 0.01 mL; $V_{Extraction}$: sample extract volume, 1.01 mL; $V_{Total\ Sample}$: the volume of adding Reagent Ⅰ and Ⅱ, 0.202 mL; T: reaction time, 2 min; W: sample weight, g; N: total number of cells, calculated in units of ten thousand.

B. Microglass cuvette calculation formula

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

Typical Data

Take 0.1 g mouse brain tissue, then follow the determination steps, and measure with 96-well plate:

$\Delta A_{Supernatant}=A_2-A_1$=0.773-0.701=0.072; $\Delta A_{Precipitate}=A_2-A_1$=0.714-0.627=0.087;

Calculate the CS activity according to the weight of the sample:

Total CS (U/g weight)=$CS_{Supernatant}+CS_{Precipitate}$=1,782.35×$\Delta A_{Supernatant}$÷W+356.47×$\Delta A_{Precipitate}$÷W=1,782.35×0.072÷0.1+356.47×0.087÷0.1=1,593.42 U/g.

Precautions

1. All samples and reagents should be on ice to avoid denaturation and deactivation.
2. 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.
3. Please to extract CS from fresh samples in order to ensure the enzyme activity.

Recommended Products

Catalog No.	Product Name
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit
KTB1250	CheKine™ Micro Mitochondrial Isocitrate Dehydrogenase (ICDHm) Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase (SDH) Activity Assay Kit
KTB1240	CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.