



CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit

Cat #: KTB1023

Size: 48 T/96 T

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|---|--|------------|--------------------------------------|
|  | 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°C 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 Krebs' 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 | 50 mL | 100 mL | 4°C |
| Reagent I | 10 mL | 20 mL | 4°C |
| Reagent II | 0.75 mL | 1.5 mL | -20°C, protected from light |
| Reagent III | 15 mL | 30 mL | 4°C |
| Reagent IV | 1 | 1 | 4°C, protected from light |
| Reagent V | 1 | 1 | -20°C, protected from light |
| Reagent VI | 1 | 1 | -20°C, 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
- 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°C.

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

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

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

Working Reagent: Before use, resolve Reagent IV and Reagent V with 11 mL Reagent III for 48 T; resolve Reagent IV and Reagent V with 22 mL Reagent III for 96 T. The remaining reagents should be stored at -20°C and protected from light after aliquoting to avoid repeated freezing and thawing.

Working Reagent VI: Before use, add 0.5 mL deionized water to fully dissolve for 48 T; add 1 mL deionized water to fully dissolve for 96 T. The remaining reagents should be stored at -20°C 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°C for one month.

Processed samples must be assayed immediately.

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 μ L Reagent II, homogenize on ice. Centrifuge at 600 g for 5 min at 4°C. 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°C, 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 μ L Reagent I and 2 μ L Reagent II to the precipitate collected in step 2, mix well, and use it to detect the activity of CS in the next step.

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°C (mammal) or 25°C (other species) water bath.
3. Add 10 μ L of sample, 220 μ L of Working Reagent, and then 10 μ L of Working Reagent VI 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.

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°C (mammal) or 25°C (other species).

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

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

$$\text{Total CS (U/g weight)} = CS_{\text{Supernatant}} + CS_{\text{Precipitate}} = \mathbf{1,782.35 \times \Delta A_{\text{Supernatant}} \div W + 356.47 \times \Delta A_{\text{Precipitate}} \div W}$$

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°C (mammal) or 25°C (other species).

$$CS (U/10^4 \text{ cells}) = [\Delta A \times V_{\text{Total}} \div (\epsilon \times d) \times 10^9] \div (V_{\text{Sample}} \div V_{\text{Total Sample}} \times 500) \div T = 0.713 \times \Delta A$$

Where: $\Delta A_{\text{Supernatant}}$: OD value of supernatant; $\Delta A_{\text{Precipitate}}$: 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; $V_{\text{Extraction}}$: sample extract volume, 1.01 mL; $V_{\text{Total Sample}}$: the volume of adding Reagent I and II, 0.202 mL; T : reaction time, 2 min; W : sample weight, g; 500: total number of cells, 5 million.

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:

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

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

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

Precautions

1. ΔA should be between 0.01-0.3, the sample should be diluted further if $\Delta A > 0.3$.
2. All samples and reagents should be on ice to avoid denaturation and deactivation.
3. 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.
4. Please to extract CS from fresh samples in order to ensure the enzyme activity.
5. Please take protective measures when operating, because Reagent II has certain toxicity.

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.