(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Citrate Synthase (CS) Activity Assay Kit

Catalog No.: BC00132

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Citrate Synthase (CS) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue
Assay Type	Quantitative
Detection Instrument	Microplate reader (412 nm)

Product Introduction

Citric acid synthetase, also known as citrate condensate enzyme, plays a crucial role in the tricarboxylic acid cycle (also known as the citric acid cycle) because the reaction it catalyzes is a key step. The tricarboxylic acid cycle is a ubiquitous metabolic pathway in aerobic organisms, serving as the hub and final metabolic pathway connecting the metabolism of the three major nutrients: carbohydrates, lipids, and amino acids.

The tricarboxylic acid cycle (TCA cycle), also known as the citric acid cycle or the Krebs cycle, is a ubiquitous metabolic pathway in aerobic organisms. It is named after citric acid, a compound containing three carboxyl groups, which is one of the main intermediate metabolites in this cycle. Alternatively, it can be named the Krebs cycle after its discoverer, Hans-Adolf Krebs. The TCA cycle is essential for the metabolism of three major nutrients. It is the final metabolic pathway of carbohydrates, lipids, and amino acids, and also the hub connecting the metabolism of carbohydrates, lipids, and amino acids.

Principle

In the tricarboxylic acid cycle, the reactant glucose or fatty acid is converted into acetyl-CoA. This "activated acetic acid" (one molecule of coenzyme linked to an acetyl group) breaks down in the cycle to produce the final product carbon dioxide and undergoes dehydrogenation. The proton is then transferred to the coenzymes nicotinamide adenine dinucleotide (NAD+) and flavin adenine

(FAD), resulting in NADH+ H+ and FADH2. NADH+ H+ and FADH2 then...

It continues to be oxidized in the respiratory chain into NAD+ and FAD, and produces water. This regulated "burning" generates ATP, providing energy.

The mitochondria of eukaryotes and the cytoplasm of prokaryotes are the sites of the tricarboxylic acid (TCA) cycle. It is a step in the respiratory process, but in aerobic organisms, it precedes the respiratory chain. Anaerobic organisms initially follow the same pathway, breaking down high-energy organic compounds, such as through glycolysis, but do not subsequently undergo the TCA cycle; instead, they proceed with fermentation, a process that does not require oxygen.

In the first step of the triacid cycle, catalytic reaction involves the ethyl group of acetyl-CoA combining with the ketone group of oxaloacetates to form citrate-CoA. This catalyzes subsequent high-energy sulfate bond hydrolysis, releasing coenzyme A and yielding citrate. Citrate synthase is a regulatory enzyme; its substrates, acetyl-CoA and oxaloacetate, are its activators, while NADH and succinyl-CoA are its inhibitors.

$$\text{Aceyl-CoA+Oxaloacetate+H}_2\text{O} \xrightarrow{\text{CS}} \text{Citrate+CoA-SH}$$

$$\text{CoA-SH} \xrightarrow{\text{DTNB}} \text{CoAS-S-TNB+TNB}$$

Components

Components	Size	Storage
Reagent 1	Liquid 120 mL × 1 bottle	Store at 4℃
Reagent 2	Liquid 20 mL × 1 bottle	Store at 4℃
Reagent 3	Powder × 1 tube	Store at 4℃ away from light
Reagent 4	Liquid 15 mL × 1 bottle	Store at 4℃
Reagent 5	Liquid 13 mL × 1 bottle	Store at 4℃ away from light
Reagent 6	Liquid 13 mL × 1 bottle	Store at 4℃
Standards	Powder × 1 tube	Store at 4℃

Consumables 1	1 plate (96 wells)	RT
Consumables 2	2 pieces	RT

Storage

This kit is stored under the specified conditions upon receipt and has a shelf life of 6 months.

Preparation before the experiment

Sample processing

Method 1: Liquid nitrogen grinding

1. Sample preparation : Take out fresh or frozen tissue (thaw at 4℃ or room temperature), rinse it with rinsing solution, blot dry with filter paper, accurately weigh 100-300mg of tissue, add liquid nitrogen, and quickly grind the tissue into powder with a grinding rod for later use.
2. Preparation of 10% test suspension: Weigh the ground tissue powder into a centrifuge tube (1.5 mL or 2 mL), and add 9 times the volume of extraction solution at a weight (g): volume (mL) ratio of 1:9. Vortex vigorously for 30 seconds, with a 2-minute interval, repeating the operation 2-3 times to prepare the 10% test suspension. Return to the ice bath to warm up.
3. Centrifugation : Place the centrifuge tube containing 10% of the test suspension in a 4℃ benchtop centrifuge and centrifuge at 10,000 rpm for 10 minutes.
4. Preparation of 10% supernatant: Remove the centrifuge tube and place it in an ice bath. Carefully aspirate the 10% supernatant to be tested and transfer it to a new centrifuge tube (1.5 mL or 2 mL) for testing. At the same time, use Coomassie Brilliant Blue reagent (or BCA reagent) to determine the protein concentration (BCA00006).

Method 2: Mechanical homogenization (if liquid nitrogen operation is not available)

1. Preparation of 10% test suspension : Take out fresh or frozen tissue (thaw at 4℃ or room temperature), rinse it with rinsing solution, and blot dry with filter paper; accurately weigh the tissue, add 9 times the volume of extraction solution at a ratio of weight (g): volume (mL) =

1:9, and mechanically homogenize under ice-water bath conditions to prepare 10% test suspension.

2. Preparation of 10% test supernatant: Centrifuge at 10,000 rpm for 10 minutes, and collect the supernatant to obtain the 10% test supernatant. Simultaneously, determine the tissue protein concentration (BCA00006) using Coomassie Brilliant Blue reagent (or BCA reagent).

Operation process

1. Instrument preparation: Set the microplate reader to the wavelength to be measured (412nm) and preheat it for 30 minutes to ensure that all parameters are in a relatively stable state for use.
2. Reagent preparation: Take out the frozen reagents (substrate solution, colorimetric reagent) and the refrigerated reagents (buffer solution, negative control solution), and equilibrate to room temperature before use.
3. Sample preparation: Place the sample to be tested in an ice bath to allow it to reach temperature equilibrium before testing.
4. Reaction steps (96-well plate operation):
   (1) Take a clean, sterile 96-well ELISA plate, place it horizontally on the test bench, and label the sample to be tested as U well (or negative control well, O well).
   (2) Add 190 μL of reagent one (buffer solution) to the U well (or O well) of a 96-well microplate;
   (3) Add 25 μL of reagent 2 (substrate solution) to the above U well (or O well);
   (4) Add 25 μL of reagent three (color developer) to the above U wells (or O wells);
   (5) Add the above reagents to the 96-well microplate, shake gently to mix, and incubate at 37° C for 3-5 minutes.
   (6) Remove the 96-well microplate containing the pre-warmed reagent and place it horizontally on the experimental platform;
   (7) Add 10 μL of the sample to be tested to the U well (or 10 μL of the negative control

solution to the O well) (the sample to be tested must be clear; if the sample is reconstituted after freezing, centrifuge to precipitate the protein before use, depending on the situation).

(8) Gently shake the ELISA plate to ensure that the sample to be tested (or the negative control solution) is fully in contact with the pre-warmed reagent; then transfer the 96-well ELISA plate into the microplate reader and measure the absorbance (OD) value of the sample to be tested at a wavelength of 412 nm, which is recorded as AU_0 (AO_0 for the negative control well).

(9) Transfer the 96-well microplate to a 37°C incubator (or a 37°C microplate reader) and incubate for 15 minutes.

(10) The 96-well microplate was then transferred to a microplate reader and the absorbance (OD) value of the sample was measured at a wavelength of 412 nm and recorded as AU_{15} (the negative control well was AO_{15}).

(11) Test sample value (U well): Absorbance of test sample AU_{15} - Absorbance of test sample AU_0. [Negative control value (O well): AO_{15} - AO_0]

Reagents	Negative control well	Sample well
Reagent 1 (μL)	190	190
Reagent 2 (μL)	25	25
Reagent 3 (μL)	25	25
Shake gently to mix, then incubate at 37°C for 3–5 minutes.		
Negative control solution (μL)	10	--
Sample to be tested (μL)	--	10
Mix thoroughly (or vortex to mix the 96-well plate) and place in a microplate reader to measure the initial absorbance A_0 at a wavelength of 412 nm. Transfer to a 37°C incubator (or a 37°C water bath) for 15 min, then immediately place in the microplate reader to measure the absorbance A_{15}. Calculate $\Delta A = A_{15} - A_0$.		

Result calculation

$$\text{CS activity (U/mgprot)} = \frac{\Delta AU - \Delta A0}{13.6 \times 10^{-3} \times d \times T} \times N_1 \div Cpr \times N$$

Note: AU represents the absorbance value of the measurement well, AO represents the absorbance value of the blank well; 13.6×10^{-3} is the micromolar extinction coefficient;

Annotation

T: Reaction time, 15 minutes;

d : Colorimetric path length, cm ;

N_1 : Dilution factor of the system, 25 ;

N : Dilution factor of the sample before testing ;

Cpr : Sample protein concentration, mgprot/mL (prot refers to protein).

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.