Citric acid (CA) Content Assay Kit

Note: The reagents have been changed, so please be aware of and follow this instruction strictly.

Detection equipment: Spectrophotometer/Microplate reader

Cat No: BC2155

Size: 100T/96S

Components:

Reagent I: Liquid 120 mL×1, store at 2-8℃.

Reagent II: Liquid 22 mL×1, store at 2-8℃.

Reagent III: Liquid 0.25 mL×1, store at -20℃. It is a volatile reagent, seal it as soon as possible after use, and store it at -20°C.

Reagent IV: Powder×2, store at room temperature. Before use, take 1 bottle and add 1.5 mL of Reagent I, dissolve it fully, and store the unused reagent at 2-8℃ for 2 weeks.

Reagent V: Liquid 3 mL×1, store at 2-8℃℃ and protect from light.

Standard: Liquid 1 mL×1,2 μmol/mL citric acid standard solution.

Description:

CA is a common organic acid in organism and an important food flavor substance. In addition, CA is the product of the first step of the tricarboxylic acid cycle. Under acid condition, Cr^{6+} is reduced to Cr^{3+} by citric acid, which have a characteristic absorption peak at 545 nm. The content of citric acid in the sample can be calculated by measuring the increase of the absorption value at 545 nm.

Required but not provided:

Spectrophotometer/plate reader, cryogenic centrifuge, water bath, adjustable pipette, micro glass cuvettes/96-well plate, mortar/homogenizer, ice and distilled water.

Operation procedure:

I. Extraction of citric acid from samples

1. Extraction of citric acid from liquid sample: take 0.1 mL of liquid and add 0.9 mL of Reagent I, mix well. Centrifuge at 11000 ×g for 10 minutes at 4℃, take the supernatant and place it on.(If the measurement value is low, you can adjust the volume ratio of liquid sample and reagent I, such as (0.2mL liquid sample +0.8mL reagent I) or (0.5mL liquid sample +0.5mL reagent I)..
2. Tissue: Homogenize in an ice bath at a ratio of 1:5~10 tissue mass (g) to Reagent I volume (mL) (it is recommended that 0.1g of tissue be weighed and 1mL of Reagent I be added). Centrifuge at 10,000g for 10min at 4℃, take the supernatant and put it on ice for measurement.
3. Bacteria or cultured cells: collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant; according to the ratio of the number of bacteria or cells (10^4): the volume of reagent I (mL)

is 500~1000:1 (it is recommended that 5 million bacteria or cells add 1mL of reagent I), ultrasonic crushing of bacteria or cells (ice bath, power 200W, ultrasonic 3s, 10s intervals, repeat for 30 times); 10,000g centrifugation at 4 ℃ for 10min, take the supernatant, and put it on the ice to be measured.

4. Mitochondrial citric acid extraction: weigh about 0.1g of tissue, add 1mL of Reagent I, grind thoroughly on ice, centrifuge at 600g at 4℃ for 5min; take the supernatant into another EP tube, centrifuge at 11000g at 4℃ for 10min, discard the supernatant (this supernatant can be used for the determination of cytoplasmic CA content); add 200μL of Reagent II and 2μL of Reagent III into the precipitate, suspend and dissolve thoroughly. Add 200μL of Reagent II and 2μL of Reagent III to the precipitate, suspend and dissolve thoroughly, centrifuge at 11000g for 10min at 4℃, and put the supernatant on ice.

5. Determination procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes,spectrophotometer adjust wavelength to 545 nm and spectrophotometer set zero with distilled water.
2. Preheat the Reagent I in water bath at 30℃ for more than 30 minutes.
3. Add the corresponding reagent into the 1.5 mL EP tube according to the following table.

Reagent name(μL)	Black tube (B)	Test tube(T)	Standard tube (S)
Distilled water	20	-	-
Supernatant	-	20	-
Standard	-	-	20
Reagent I	140	140	140
Reagent IV	20	20	20
Reagent V	20	20	20
After fully mixing, leave it for 30 minutes at room temperature, measure the absorbance at 545 nm, and record it as A_B, A_T, A_S. Blank tube and standard tube only need to measure 1-2 times.			

III. Calculation:

1. Calculate according to the volume of liquid sample:

The content of citric acid(μmol/mL) = $[C_S\times(A_T-A_B)\div(A_S-A_B)]\times F$

$= 20\times(A_T-A_B)\div(A_S-A_B)$

2. Calculated according to fresh weight of tissue:

The content of citric acid(μmol/g mass) = $[C_S\times(A_T-A_B)\div(A_S-A_B)]\times V_T\div W$

$= 2\times(A_T-A_B)\div(A_S-A_B)\div W$

3. Calculated according to number of cells/bacteria:

The content of citric acid(μmol/10^4 cell) = $[C_S\times(A_T-A_B)\div(A_S-A_B)]\times V_T\div N$

$= 2\times(A_T-A_B)\div(A_S-A_B)\div N$

4. Calculated according to the content of mitochondrial protein:

The content of citric acid(μmol/mg prot) = $[C_S\times(A_T-A_B)\div(A_S-A_B)]\times V_S\div(Cpr\times V_S)$

$=2\times(A_T-A_B)\div(A_S-A_B)\div Cpr$

C_S: Standard concentration, 2 μmol/mL;

F: Sample dilution times, (0.1 mL sample + 0.9 mL Reagent I)÷0.1 mL sample = 10.

V_T: Total volume of supernatant, 1.0 mL;

W: Sample mass, g.

N: number of cells/bacteria, in tens of thousands;

V_S: Sample volume, 0.02mL;

Cpr: Protein concentration of supernatant, mg/mL.

Note:

1、Sample processing and other processes need to be carried out on ice.

2、Reagent V is a carcinogenic substance. During the experiment, gloves should be worn to avoid splashing reagent V on the skin.

3、Citric acid reagent I can not be used for protein content determination, if you need to determine the protein content, you need to take another tissue for determination.If there are obvious small black particles after 30 minutes of reaction, it is a normal phenomenon, the sample should be diluted and then measured.

4、If there are obvious black particles after 30min reaction, it is a normal phenomenon, need to dilute the sample and then measure.

5、If the absorbance value of the sample is more than 0.5, it is recommended to dilute the sample with reagent I for measurement..

Experimental example:

1. take 0.1065g of bamboo leaves add 1mL of reagent I, fully ground on ice, 11000g 4 ℃ centrifugation 10min, take the supernatant In accordance with the steps of the determination of operation, measurement and calculation of ΔAt = At - Ab =0.185-0.109=0.076, ΔAs = As- Ab = 0.292- 0.109=0.183, the sample mass was calculated according to the sample mass, the sample mass was calculated as follows: Calculated from sample mass:

Citric acid content (μmol/g mass) = 2 × ΔAt ÷ ΔAs ÷ W =7.80 μmol/g mass.

2. Take 0.1094g of rabbit kidney tissue and add 1mL of reagent 1, grind it thoroughly on ice, centrifuge it at 11000g for 10min at 4℃, take the supernatant and operate according to the assay procedure, and then calculate ΔAt = At - Ab=0.270-0.109=0.161,ΔAs = As- Ab =0.292- 0.109= 0.183, calculated according to the mass of sample. Calculated from sample mass:

Citric acid content (μmol/g mass) = 2 × ΔAt ÷ ΔAs ÷ W =16.08 μmol/g mass.

3. Take 0.1mL of rabbit serum, add 0.9mL of reagent 1, mix thoroughly, centrifuge at 11000g for 10min at 4℃, take the supernatant and operate according to the steps of the assay, and then measure the ΔA content (μmol/g mass). ΔAt = At - Ab =0.174-0.109=0.065, ΔAs = As- Ab =0.292-0.109 =0.183, calculated according to the volume of liquid sample:

Citric acid content (μmol/mL) = 20 x ΔAt ÷ ΔAs =7.10 μmol/mL..

Recent Product Citations:

[1] Meixi Peng, Dan Yang, Yixuan Hou, et al. Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKP and CS enhances hypoxic breast cancer cell invasion and metastasis. Cell Death and Disease. March 2019;(IF5.959)

[2] Luo M,Luo Y, Mao N,et al. Cancer-Associated Fibroblasts Accelerate Malignant Progression of Non-Small Cell Lung Cancer via Connexin 43-Formed Unidirectional Gap Junctional Intercellular Communication. Cellular Physiology and Biochemistry. November 2018;

[3] Zhou Z, Duan Y, Zhou M. Carbendazim-resistance associated β2-tubulin substitutions increase deoxynivalenol biosynthesis by reducing the interaction between β2-tubulin and IDH3 in Fusarium graminearum [J]. Environmental microbiology, 2019.

Related Products:

BC0710/BC0715	α-Ketoglutarate Dehydrogenase(α-KGDH) Activity Assay Kit
BC0950/BC0955	Succinate Dehydrogenase(SDH) Activity Assay Kit
BC0380/BC0385	Pyruvate Dehydrogenase(PDH) Activity Assay Kit

Technical Specifications:

The detection limit: 52.09 μmol/L

Linear range: 62.5-6000 μmol/L

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com