

Citric acid(CA) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer

Cat No: NA0717

Size: 50T/48S

Components:

Reagent I: 90 mL×1, store at 4°C.

Reagent II: 10 mL×1, store at 4°C.

Reagent III: 0.1 mL×1, store at -20°C.

Reagent IV: Powder×1, store at room temperature. Add 5 mL of Reagent I and fully dissolve when the solution will be used.

Reagent V: 5 mL×1, store at 4°C and protect from light.

Standard: 1 mL×1, 1 mmol/mL citric acid standard solution, store at 4°C. Dilute it to obtain 250 μmol/L citric acid standard solution when the solution will be used.

Description:

Citric acid (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:

Low temperature centrifuge, water bath, transferpeltor, spectrophotometer, mortar/homogenizer, 1 mL glass cuvette, 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°C, take the supernatant for test.

2. Extraction of citric acid from tissue: weigh about 0.1 g of tissue, add 1 mL of Reagent I, grind it on ice fully, centrifuge it at 11000 ×g for 10 minutes at 4°C, take the supernatant for test.

3. Extraction of citric acid from mitochondria: weigh about 0.1 g of tissue, add 1 mL of Reagent I, grind it on ice fully, centrifuge it at 4°C for 5 minutes at 600 ×g, take the supernatant to another EP tube. Centrifuge it at 11000 ×g for 10 minutes at 4°C, discard the supernatant (this supernatant can be used for the determination of CA content in cytoplasmic). Add 200 μL of Reagent II and 2 μL of Reagent III to the precipitation, fully suspend and dissolve it. Centrifugate it at 11000 ×g for 10 minutes at 4°C, take the

supernatant for test.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 545 nm and set zero with distilled water.
2. Preheat the Reagent I in water bath at 30°C 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	100	-	-
Supernatant	-	100	-
Standard	-	-	100
Reagent I	700		
Reagent IV	100		
Reagent V	100		

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 .

III. Calculation:

1. Calculate according to the volume of liquid sample:

$$\begin{aligned}\text{The content of citric acid (mmol/L)} &= [C_S \times (A_T - A_B) \div (A_S - A_B)] \times F \\ &= 2.5 \times (A_T - A_B) \div (A_S - A_B)\end{aligned}$$

C_S : Standard concentration, 250 μmol/L = 0.25 mmol/L;

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

2. Calculated according to fresh weight of tissue:

$$\begin{aligned}\text{The content of citric acid (μmol/g FW)} &= [C_S \times (A_T - A_B) \div (A_S - A_B)] \times V_T \div W \\ &= 0.25 \times (A_T - A_B) \div (A_S - A_B) \div W\end{aligned}$$

C_S : Standard concentration, 250 μmol/L;

V_T : Total volume of supernatant, 1.0 mL = 0.001 L;

W: Sample mass, g.

3. Calculated according to the content of mitochondrial protein:

$$\begin{aligned}\text{The content of citric acid (μmol/mg prot)} &= [C_S \times (A_T - A_B) \div (A_S - A_B)] \times V_S \div (C_{pr} \times V_S) \\ &= 0.25 \times (A_T - A_B) \div (A_S - A_B) \div C_{pr}\end{aligned}$$

C_S : Standard concentration, 250 μmol/L = 0.25 μmol/mL;

V_S : Sample volume, 100 μL = 0.1 mL;

C_{pr} : Protein concentration of supernatant, mg/mL.

Note:

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

2. Reagent V is a carcinogen. During the experiment, gloves should be worn to avoid Reagent V splashing on the skin.
3. The extract solution of citric acid cannot be used for measuring the content of protein. If the content of protein needs to be determined, please take another tissue and use BCA kit of our company for determination.
4. 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.
5. If the sample absorbance value is greater than 0.8, it is recommended to dilute the sample with Reagent I and then determine it.

Experimental example:

1. Take 0.1g of Ilex, add 1 mL of Reagent I, grind it on ice, centrifuge it at 11000g and 4°C for 10 min, dilute the supernatant 4 times, and then operate according to the determination steps. Use 96 well plate to measure and calculate $A_T = 0.343$, $A_B = 0.107$, $A_S = 0.162$

Citric acid content ($\mu\text{mol/g mass}$) = $0.25 \times (A_T - A_B) \div (A_S - A_B) \div W \times 4 = 42.91 \mu\text{mol/g mass}$.

2. Take 0.1g of rat muscle tissue, add 1 mL of Reagent I, grind it fully on ice, centrifuge at 11000g and 4°C for 10 min, dilute the supernatant 4 times and operate according to the determination steps. Use 96 well plate to measure and calculate $A_T = 0.645$, $A_B = 0.107$, $A_S = 0.162$.

Citric acid content ($\mu\text{mol/g mass}$) = $0.25 \times (A_T - A_B) \div (A_S - A_B) \div W \times 4 = 97.82 \mu\text{mol/g mass}$.

3. Take 0.1 mL of mouse serum, add 0.9 mL of Reagent I, mix well, centrifuge at 11000g and 4°C for 10 min, take 4 times diluted supernatant and operate according to the determination steps. Use 96 well plate to measure and calculate $A_T = 0.347$, $A_B = 0.107$, $A_S = 0.162$.

Citric acid content (mmol/L) = $2.5 \times (A_T - A_B) \div (A_S - A_B) \times 4 = 43.64 \text{ mmol/L}$.

Recent Product Citations:

[1] Meixi Peng, Dan Yang, Yixuan Hou, et al. Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKFB3 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 β 2-tubulin substitutions increase deoxynivalenol biosynthesis by reducing the interaction between β 2-tubulin and IDH3 in *Fusarium graminearum* [J]. *Environmental microbiology*, 2019.

Related Products:

- NA0812/NA0570 α -Ketoglutarate Dehydrogenase(α -KGDH) Activity Assay Kit
 NA0799/NA0558 Succinate Dehydrogenase(SDH) Activity Assay Kit
 NA0837/NA0595 Pyruvate Dehydrogenase(PDH) Activity Assay Kit

Technical Specifications:

The detection limit: 19.93 $\mu\text{mol/L}$

Linear range: 31.25-4000 $\mu\text{mol/L}$