# Citric acid(CA) Content Assay Kit

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

**Detection equipment:** Spectrophotometer/Microplate reader

**Cat No:** NA0476

**Size:** 100T/96S

**Components:**

Reagent I: 60 mL×1, store at 4℃.

Reagent II: 20 mL×1, store at 4℃.

Reagent III: 0.2 mL×1, store at -20℃.

Reagent IV: Powder×1, store at room temperature. Add 2 mL of Reagent I and fully dissolve when the solution will be used.

Reagent V: 2 mL×1, store at 4℃ and protect from light.

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

**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:**

Low temperature centrifuge, water bath, transferpettor, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, 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 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 at 11000 ×g for 10 minutes at 4℃, 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℃ for 5 minutes at 600 ×g, take the supernatant to another EP tube. Centrifuge at 11000 ×g for 10 minutes at 4℃, 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 at 11000 ×g for 10 minutes at 4℃, take the supernatant for test.

**II. Determination procedure**

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 545 nm and 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 | | |
| Reagent IV | 20 | | |
| Reagent V | 20 | | |
| After fully mixing, leave it for 30 minutes at room temperature, measure the absorbance at 545 nm, and record it as $A_B$, $A_T$, $A_S$. | | | |

**III. Calculation:**

1. Calculate according to the volume of liquid sample:

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

$= 2.5 \times (A_T-A_B) \div (A_S-A_B)$

$C_S$: 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:

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

$= 0.25 \times (A_T-A_B) \div (A_S-A_B) \div W$

$C_S$: 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:

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

$= 0.25 \times (A_T-A_B) \div (A_S-A_B) \div Cpr$

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

$V_S$: Sample volume, 20 μL= 0.02 mL;

Cpr: 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, places 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.

**Experimental example:**
1. Take 0.1g of Ilex, add 1 mL of Reagent I, grind it on ice, centrifuge it at 11000g and 4℃ for 10 min, dilute the supernatant 4 times, and then operate according to the determination steps. Use 96 well plate to measure and calculate $A_T$= 0.220, $A_B$= 0.114, $A_S$= 0.146
Citric acid content (μmol/g mass) = 0.25 ×($A_T$-$A_B$) ÷ ($A_S$-$A_B$) ÷ W × 4 = 33.13 μmol/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℃ for 10 min, dilute the supernatant 4 times and operate according to the determination steps. Use 96 well plate to measure and calculate $A_T$ = 0.347, $A_B$ = 0.114, $A_S$ = 0.146.
Citric acid content (μmol/g mass) = 0.25×($A_T$-$A_B$) ÷ ($A_S$-$A_B$) ÷W × 4 = 72.81 μmol/g mass).
3. Take 0.1 mL of mouse serum, add 0.9 mL of Reagent I, mix well, centrifuge at 11000g and 4℃ for 10 min, take 4 times diluted supernatant and operate according to the determination steps. Use 96 well plate to measure and calculate $A_T$ = 0.201, $A_B$ = 0.114, $A_S$= 0.146.
Citric acid content (mmol/L) = 2.5 × ($A_T$-$A_B$) ÷ ($A_S$-$A_B$) × 4 = 27.19 mmol/L.

**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：**

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|---|---|
| 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: 52.09 μmol/L
Linear range: 62.5-6000 μmol/L