PRODUCT INFORMATION & MANUAL

Citric Acid Assay Kit (Colorimetric)

NBP3-25777

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

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Citric Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25777

Method: Colorimetric method

Specification: 100Assays (Can detect 96 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.05 mmol/L

Detection range: 0.05-5.0 mmol/L

Average intra-assay CV (%): 4.2

Average inter-assay CV (%): 5.4

Average recovery rate (%): 96

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure citric acid (CA) content in tissue, mitochondria and other liquid samples.

▲Background

In biochemistry, citric acid is an intermediate in the citric acid cycle and plays an important role in metabolism. Citric acid levels in blood and urine are affected by factors such as age, gender, diet, citric acid precursors, and parathyroid hormone and sex hormones.

▲Detection principle

In acidic condition, Cr (Ⅵ) will be reduced to Cr^{3+}, Cr^{3+} reacts with citric acid. And the product has a characteristic absorption peak at 545 nm, therefore the content of citric acid in sample can be calculated by measuring the absorbance value at 545 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	45 mL × 4 vials	2-8℃，12 months
Reagent 2	Lysis Buffer	20 mL × 1 vial	2-8℃，12 months
Reagent 3	Reducing Agent	Powder × 1 vial	2-8℃，12 months, shading light
Reagent 4	Chromogenic Agent	15 mL × 1 vial	2-8℃，12 months, shading light
Reagent 5	1 mmol/L CA Standard	2 mL × 1 vial	2-8℃，12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (545 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key point of the assay

Preheat reagent 1 at 30℃ water bath for 30 min before use.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 3 working solution

Dissolve a vial of reagent 3 with 20 mL of reagent 1 fully. The prepared solution can be stored at 2-8℃ for 7 days.

2. Preparation of 0.25 mmol/L standard application solution

Dilute 1 mmol/L CA standard with double distilled water for 4 times. Prepare the fresh solution before use.

▲Sample preparation

Sample requirements

The samples could not contain chelating agents such as EGTA and EDTA, or reductive substances such as DTT and mercapto ethanol.

Extraction of citric acid

1. Extraction of citric acid in liquid samples: take 0.1 mL of liquid sample and add 0.9 mL of reagent 1, mix fully. Centrifuge at 11000 g for 10 min at 4℃ , then take the supernatant and stand on ice for measurement.
2. Extraction of citric acid in tissue sample: take 0.1 g tissue, add 0.9 mL of reagent 1, then homogenize the sample in ice water bath. Centrifuge at 11000 g for 10 min at 4℃ , then take the supernatant and stand on ice for measurement.
3. Extraction of citric acid in mitochondria: take 0.1 g tissue, add 0.9 mL of reagent 1, then homogenize the sample in ice water bath. Centrifuge at 600 g for 10 min at 4℃ , then take the supernatant to another EP tube and centrifuge at 11000 g for 10 min at 4℃ , discard the supernatant (This supernatant can be used for the determination of citric acid content in

cytoplasmic). Add 200 μL of reagent 2 and dissolve the precipitate fully with vortex mixer. Centrifuge at 11000 g for 10 min at 4℃ , then take the supernatant and stand on ice for measurement.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.05-5.0 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	4-6
10% Mouse kidney tissue homogenate	2-4
10% Rat kidney tissue homogenate	1
10% Mouse heart tissue homogenate	2-4
10% Mouse brain tissue homogenate	1
10% Mouse liver tissue homogenate	1

Note:The diluent is reagent 1.

Assay protocol

▲Detailed operating steps

1. Blank tube: Take 100 μL of double distilled water to the 1.5 mL EP tube.
   Standard tube: Take 100 μL of 0.25 mmol/L standard application solution to the 1.5 mL EP tube.
   Sample tube: Take 100 μL of sample supernatant to the 1.5 mL EP tube.
2. Add 700 μL of reagent 1 to each tube.
3. Add 100 μL of reagent 3 working solution to each tube.
4. Add 100 μL of reagent 4 to each tube.
5. Mix fully with vortex mixer and stand for 30 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 545 nm wavelength with 1 mL quartz cuvette.

▲Summary operation table

	Blank tube	Sample tube	Standard tube
Double distilled water (μL)	100		
Sample supernatant (μL)		100	
0.25 mmol/L Standard application solution (μL)			100
Reagent 1 (μL)	700	700	700
Reagent 3 working solution (μL)	100	100	100
Reagent 4 (μL)	100	100	100
Mix fully and stand for 30 min at room temperature. Measure the OD values at 545 nm with 1 mL quartz cuvette.			

▲Calculation

1. Liquid sample

$$\text{CA content（ mmol/L ）} = (\Delta A_1 \div \Delta A_2) \times c \times f \times 10$$

2. Tissue sample

$$\text{CA content（ μmol/g fresh weight ）} = (\Delta A_1 \div \Delta A_2) \times c \times f \div (m / V)$$

3. Mitochondria samples

$$\text{CA content（ μmol/mg prot ）} = (\Delta A_1 \div \Delta A_2) \times c \div C_{pr}$$

Note:

ΔA_1: OD_{Sample} - OD_{Blank}

ΔA_2: $OD_{Standard}$ - OD_{Blank}

c: Concentration of standard (0.25 mmol/L).

f: Dilution factor of sample before test.

10: Dilution factor of liquid sample in citric acid extraction step (0.1 mL of sample + 0.9 mL of reagent 1).

m: The weight of tissue sample (0.1 g).

V: The volume of extracting solution (0.9 mL).

Cpr: Protein concentration of sample (mgprot/mL).

Appendix I Data

▲Example analysis

Dilute human serum with normal saline (0.9% NaCl) for 5 times, take 100 μL of diluted sample and carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.344, the average OD value of the blank is 0.119, the average OD value of the standard is 0.173, and the calculation result is:

$$\text{CA content (mmol/L)} = (0.344 - 0.119) \div (0.173 - 0.119) \times 0.25 \times 5 \times 10$$
$$= 52.083 \text{ mmol/L}$$

Appendix II References

1. Dixon T F, Perkins H R. Citric acid and bone metabolism[J]. Biochemical Journal, 1952, 52(2): 260-265.
2. Chang T S, Freeman S. Citric acid and its relation to serum and urinary calcium[J]. Am J Physiol, 1950, 160(2): 330-340.