PRODUCT INFORMATION & MANUAL

Citric Acid Assay Kit (Colorimetric)

NBP3-25930

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

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Citric Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25930

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.06 mmol/L

Detection range: 0.06-2.0 mmol/L

Average intra-assay CV (%): 4.0

Average inter-assay CV (%): 4.0

Average recovery rate (%): 95

- 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 animal tissue, serum (plasma) and mitochondria 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	Extracting Solution	60 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Lysis Buffer	20 mL × 1 vials	2-8℃ , 12 months
Reagent 3	Reducing Agent	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	1.2 mL × 2 vials	2-8℃ 12 months, shading light
Reagent 5	2 mmol/L CA Standard	2 mL × 1 vial	2-8℃ 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader (535-555 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents

Double distilled water

⚠ 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

There should be no bubbles in the wells of the microplate when measuring the OD value.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.

2. Preparation of reagent 3 working solution

Dissolve a vial of reagent 3 with 5 mL of reagent 1 fully. The prepared solution can be stored at 2-8℃ with shading light for 7 days.

▲Sample preparation

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 for 1 min. 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 (0.06-2.0 mmol/L).

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

Sample type	Dilution factor
Human serum	5-20
Dog serum	5-10
Rat serum	10-20
Horse serum	5-10
Mouse plasma	5-20
10% Rat brain tissue homogenate	5-10
10% Rat liver tissue homogenate	5-20
10% Rat kidney tissue homogenate	5-10
10% Rat lung tissue homogenate	15-30
10% Mouse heart tissue homogenate	5-20

Note:The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operating steps

The preparation of standard curve

Dilute 2 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 2.0, 1.5, 1.2, 1.0, 0.8, 0.5, 0.2, 0 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	2 mmol/L Standard(μL)	Double distilled water (μL)
A	0	0	200
B	0.2	20	180
C	0.5	50	150
D	0.8	80	120
E	1.0	100	100
F	1.2	120	80
G	1.5	150	50
H	2.0	200	0

The measurement of samples

1. Standard well: Take 20 μL of standard solution with different concentrations into the corresponding wells.
   Sample well: Take 20 μL of sample into the corresponding wells.
2. Add 140 μL of reagent 1 to each well.
3. Add 20 μL of reagent 3 working solution to each well.
4. Add 20 μL of reagent 4 to each well.
5. Mix fully with microplate reader for 5 s and stand at room temperature for 30 min. Measure the OD values of each well at 545 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Sample (μL)		20
Standards solution with different concentrations (μL)	20	
Reagent 1 (μL)	140	140
Reagent 3 working solution (μL)	20	20
Reagent 4 (μL)	20	20
Mix fully and stand at room temperature for 30 min. Measure the OD values at 545 nm.		

Note: If there is obvious turbidity in the well after reaction for 30 min, it is a normal phenomenon, dilute the sample and test again.

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.
The standard curve is: y= ax + b.

1. Serum (plasma) Liquid sample

CA content（ mmol/L ）= (ΔA - b) ÷ a × f ×10

2. Tissue sample

CA content（ μmol/g wet weight ）= (ΔA - b) ÷ a × f ÷ (m / V)

3. Mitochondria samples

CA content（ mmol/gprot ）= (ΔA - b) ÷ a ÷ C_{pr} × f

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0);

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve;

ΔA: OD_{Sample} - OD_{Blank};

10: Dilution factor of liquid sample in extraction step (0.1 mLof sample + 0.9 mL of reagent 1);

f: Dilution factor of sample before test;

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

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

C_{pr}: Protein concentration of sample (gprot/L).

Appendix I Data

▲Example analysis

Take 0.1 mL of human serum and add 0.9 mL of reagent 1, mix fully and extracted by vortex for 1 min. After centrifugation, the supernatant was diluted for 20 times with reagent 1, and take 20 μL to the corresponding well and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0672 x + 0.0009, the OD value of the sample is 0.099, the OD value of the blank is 0.071, and the calculation result is:

CA content (mmol/L) =（0.099-0.071-0.0009）÷0.0672×20×10

= 80.65 mmol/L