Total Carbonyl Colorimetric Assay Kit

Catalog No: E-BC-K171-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 86 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.94 μg/mL

Detection range: 0.94-45 μg/mL

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

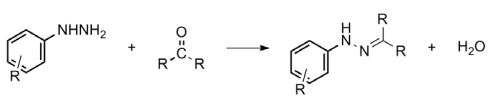
This kit can be used for detection of total carbonyl content in serum, plasma and tissue samples.

▲Background

Carbonyl is an organic functional group formed by carbon and oxygen. Carbonyl groups (aldehydes and ketones) can be introduced into biomolecules through oxidation. The production of carbonyl groups is considered to be the indirect evidence of the oxidation of biomolecules. The measurement of carbonyl content is helpful to the study of physiology and biochemistry.

▲Detection principle

Carbonyl can react with 2,4-dinitrophenylhydrazine and produce a kind of reddish brown hydrazone compounds, which has a specific absorbance peak at 370 nm. The content of carbonyl can be calculated according to the absorbance value.



▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Working Solution	30 mL × 1 vial	2-8℃ , 6 months, shading light
Reagent 2	100 μg/mL Standard	2 mL × 1 vial	2-8℃ , 6 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 (370 nm), Micropipettor, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

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

The supernatant of treated sample must be clarified.

Pre-assay preparation

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲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 (0.94-45 μg/mL).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	370 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

The preparation of standard curve

Dilute 100 μg/mL standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 5, 10, 20, 30, 40, 45 μg/mL.

The measurement of samples

(1) Blank tube: add 1.62 mL of double distilled water to the 2 mL EP tube.
Standard tube: add 1.5 mL of double distilled water and 0.12 mL of standard with different concentrations to the 2 mL EP tube.
Sample tube: add 1.5 mL of double distilled water and 0.12 mL of sample to the 2 mL EP tube.

(2) Add 0.25 mL of Reagent 1 and oscillate fully.

(3) Stand for 5 min at room temperature. Set the spectrometer to zero with double distilled water and measure the OD values of each tube at 370 nm with 0.5 cm optical path cuvette.

▲Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	1.62	1.5	1.5
Standard solution with different concentrations (mL)		0.12	
Sample (mL)			0.12
Reagent 1 (mL)	0. 25	0. 25	0. 25
Mix fully and stand for 5 min at room temperature. Set the spectrometer to zero with double distilled water and measure the OD values of each tube at 370 nm with 0.5 cm optical path cuvette.			

▲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. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. Serum (plasma) sample:

$$\text{Total carbonyl content } (\mu g/mL) = (\Delta A_{370} - b) \div a \times f$$

2. Tissue sample:

$$\text{Total carbonyl content } (\mu g/g) = (\Delta A_{370} - b) \div a \div c \times f$$

Note:

y: $OD_{Sample} - OD_{Blank}$.(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_{370}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

c: The content of sample = the wet weight (g) ÷ the volume of homogenized medium (mL)

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.94-45 μg/mL	Average intra-assay CV (%)	4.4
Sensitivity	0.94 μg/mL	Average inter-assay CV (%)	8.3
Average recovery rate (%)	100		

▲Example analysis

Take 10% mouse liver tissue homogenate, then dilute the supernatant with PBS for 6 times, take 0.12 mL of diluted sample, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.00366 x + 0.00354, R^2=0.99719. The average OD value of the sample is 0.445, the average OD value of the blank is 0.379, and the calculation result is:

Total carbonyl content (μg/g) = (0.445-0.379-0.00354) ÷ 0.00366 ÷ (0.1 g÷0.9 mL)× 6 =917 μg/g

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4 ℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.