Technical Manual

Total Carbonyl Colorimetric Assay Kit

- **Catalogue Code: MAES0129**
- **Size: 100 Assays**
- **Research Use Only**

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.94-45 µg/mL

Sensitivity:

0.94 µg/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use:

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

4. 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.

5. Kit Components & Storage:

Item	Specification	Storage
Working Solution	30 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard (100 μg/mL)	2 mL × 1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (370 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

The supernatant of samples must be clarified.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation:

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

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

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb water and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.94-45 μg/mL).

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 370 nm

10. Operation 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 working solution 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
Working solution (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.			

11. Calculations:

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) sample:

$$\text{Total carbonyl content (μg/mL)} = (\Delta_{370} - b) \div a \times f$$

2. Tissue sample:

$$\text{Total carbonyl content (μg/g)} = (\Delta_{370} - b) \div a \div c$$

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_{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).

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12. Performance Characteristics:

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

Analysis

Take 10% mouse liver tissue homogenate, then dilute the supernatant with PBS for 6 times, take 0.12 mL of diluted sample, 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:

$$\text{Total carbonyl content (μg/g)} = \frac{(0.445-0.379-0.00354)}{0.00366} \div (0.1\ g \div 0.9\ mL) \times 6$$

$$= 917\ \mu g/g$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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