# Technical Manual

# Total Carbonyl Colorimetric Assay Kit

- **Catalogue Code: MAES0128**
- **Size: 96T**
- **Research Use Only**

# 1. Key Features and Sample Types:

## Detection method:

Colorimetric method

## Specification:

96T

## Range:

1.29-45 µg/mL

## Sensitivity:

1.29 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 |
|---|---|---|
| **Chromogenic Agent Stock Solution** | 1.5 mL × 2 vials | 2-8°C, 6 months, avoid direct sunlight |
| **Standard (100 μg/mL)** | 1 mL × 1 vial | 2-8°C, 6 months |
| **Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (365-375 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:

1. The supernatant of sample must be clarified.

2. If the samples are frozen used, centrifuge at 10000 g for 10 min and take the supernatant for measurement.

## 7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **chromogenic agent working solution:** Dilute the chromogenic agent stock solution with double distilled water at the ratio of 1:6. Prepared the fresh solution before use. The prepared solution can be stored at 2-8°C for 7 days.

## 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°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 (1.29-45 μg/mL).

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

| Sample Type: | Dilution Factor: |
|---|---|
| Porcine serum | 1-2 |
| Human serum | 1-2 |
| Human plasma | 1-2 |
| 10% Rat kidney tissue homogenate | 3-5 |
| 10% Rat liver tissue homogenate | 3-5 |
| 10% Epipremnum aureum tissue homogenate | 1 |

**Note:** The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

## 9. Assay Protocol:

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** 370 nm

### 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.

# 10. Operation Steps:

## The preparation of standard curve

Dilute 100 μg/mL standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 40, 45 μg/mL.

## The measurement of samples

1. **Standard well:** add 24 μL of standard with different concentrations into standard wells.
   **Sample well:** add 24 μL of sample into sample wells.

2. Add 175 μL of chromogenic agent working solution to each well.

3. Mix fully for 5s with microplate reader and stand for 5 min at room temperature.

4. Measure the OD values of each well at 370 nm with microplate reader.

## Operation Table

| | Standard well | Sample well |
|---|---|---|
| **Standard with different concentrations (μL)** | 24 | |
| **Sample (μL)** | | 24 |
| **Chromogenic agent working solution (μL)** | 175 | 175 |
| Mix fully for 5s with microplate reader and stand for 5 min at room temperature. Measure the OD values of each well at 370 nm with microplate reader. | | |

# 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.
**$\Delta A_{370}$:** $OD_{Sample} - OD_{Blank}$
**f:** Dilution factor of sample before test.
**C:** The content of sample = the wet weight (g) ÷ the volume of homogenized medium (mL).

## 12. Performance Characteristics:

| | |
|---|---|
| **Detection Range** | 1.29-45 μg/mL |
| **Sensitivity** | 1.29 μg/mL |
| **Average recovery rate (%)** | 101 |
| **Average inter-assay CV (%)** | 5.0 |
| **Average intra-assay CV (%)** | 1.9 |

### Analysis

Take 24 μL of human serum, carry the assay according to the operation table.

**The results are as follows:**

Standard curve: y = 0.00623 x + 0.01228, the average OD value of the sample is 0.729, the average OD value of the blank is 0.444, and the calculation result is:

$$\text{Total carbonyl content (μg/mL)} = (0.729 - 0.444 - 0.01228) \div 0.00623$$

$$= 43.78 \text{ μg/mL}$$

## 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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