TECHNICAL MANUAL

Total Carbonyl Colorimetric Assay Kit

- **SKU CODE:** MAES0128
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure OD values at 370 nm
- ![img-0.jpeg](img-0.jpeg)

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Chromogenic Agent Stock Solution	1.5 mL x 1 vial	1.5 mL x 2 vials	1.5 mL x 10 vials	2-8°C, 12 months, shading light
Reagent 2	100 µg/mL Standard	1 mL x 1 vial	1 mL x 1 vial	1 mL x 5 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement	
	Plate Sealer	2 pieces	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (365-375 nm, optimum wavelength: 370 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer.

Reagents:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of chromogenic working solution:** For each well, prepare 175 µL of chromogenic working solution (mix well 25 µL of chromogenic agent stock solution and 150 µL of double distilled water). The chromogenic working solution can be stored at 2-8°C for 7 days.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. 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.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one month.

Tissue samples:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of samples:

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

- 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). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The supernatant of the sample must be clarified.

2. If the samples are previously frozen, centrifuge at 10,000 xg for 10 min and take the supernatant for measurement.

9. Operating steps

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 working solution to each well.

3. Mix fully for 5 seconds with microplate reader and stand for 5 minutes at room temperature.

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

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

Total carbonyl content (µg/mL) = (ΔA_370 - b) ÷ a × f

2. Tissue sample:

Total carbonyl content (µg/g) = (ΔA_370 - b) ÷ a ÷ c × f

[Note]

ΔA_370: the absolute OD (OD_{sample} - OD_{blank})

f: dilution factor of the sample before tested.

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	5.50	19.70	34.50
%CV	2.3	2.0	1.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	5.50	19.70	34.50
%CV	5.1	4.6	5.3

Recovery

Three samples of high, middle, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 101%.

Recovery

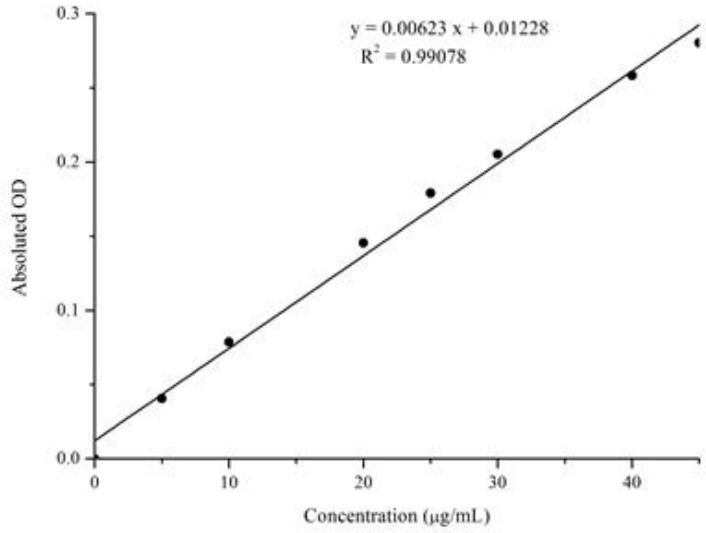
	Standard 1	Standard 2	Standard 3
Expected Conc. (µg/mL)	8.5	22.5	37.4
Observed Conc. (µg/mL)	8.6	23.2	37.0
Recovery rate (%)	101	103	99

Sensitivity

The analytical sensitivity of the assay is 1.29 µg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only.



Standard curve

Concentration (µg/mL)	Average OD	Absolute OD
0	0.454	0
5	0.495	0.041

Concentration (μg/mL)	Average OD	Absolute OD
10	0.533	0.079
20	0.600	0.146
25	0.634	0.180
30	0.660	0.206
40	0.713	0.259
45	0.735	0.281

12. Appendix II Example Analysis

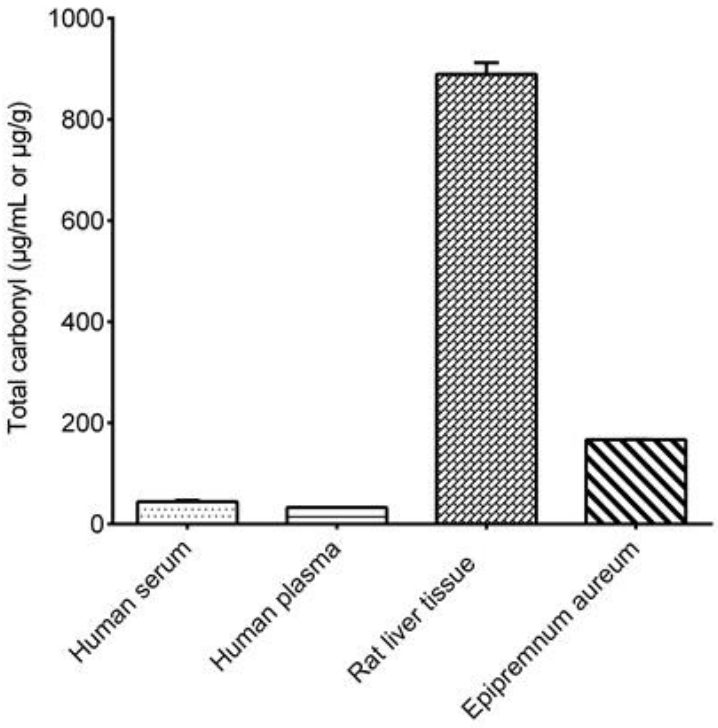
Example analysis:

Take 24 μL of human serum and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.00623x + 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:

Total carbonyl content (μg/mL) = (0.729 - 0.444 - 0.01228) ÷ 0.00623 = 43.78 μg/mL

Detect human serum, human plasma, rat kidney tissue homogenate (the content of sample is 0.111 g/mL, diluted 3 times) and epipremnum aureum tissue homogenate (the content of sample is 0.111 g/mL) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance is not within the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.