TECHNICAL MANUAL

Protein Carbonyl Colorimetric Assay Kit (Tissue And Serum Samples)

- **SKU CODE:** MAES0096
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample pretreatment
- Sample measurement
- Protein precipitation
- Washing with ethanol-ethyl acetate mixture
- Denaturation and measurement

2. Intended use

This kit can be used for detection of protein carbonyl content in serum (plasma), tissue, hydrothorax, and cell culture supernatant samples.

3. Detection principle

The content of protein carbonyl increases after oxidation, and the carbonyl group reacts with 2,4-dinitrophenylhydrazine to form a reddish brown precipitate. The absorbance can be measured at 370 nm after the precipitation is dissolved. The carbonyl content can be calculated indirectly.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Homogenate Medium	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 2	Sulfates	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	DNPH Solution	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Acid Reagent	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months
Reagent 5	Protein Precipitator	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 6	Denaturant	37.5 mL × 2 vials	50 mL × 3 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (360-385 nm, optimum wavelength: 370 nm), Vortex mixer, Micropipettor, Water bath, Incubator, Centrifuge

Reagents:

Double distilled water, deionized water, anhydrous ethanol, ethyl acetate

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of sulfates application solution:** Dissolve one vial of sulfates with 3 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 3 days protected from light.
3. **Preparation of anhydrous ethanol-ethyl acetate mixture application solution:** For each tube, prepare 1000 µL of anhydrous ethanol-ethyl acetate mixture application solution (mix well 500 µL of anhydrous ethanol and 500 µL of ethyl acetate). The anhydrous ethanol-ethyl acetate mixture application solution should be prepared fresh.

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.

Hydrothorax sample: Collect fresh hydrothorax sample into a tube containing anticoagulant, centrifuge at 10,000×g for 10 min at 4°C and take the supernatant to preserve it on ice for detection. If not detected on the same day, the sample can be stored at -80°C for one month.

Cell culture supernatant: Collect the fresh cell culture supernatant, centrifuge at 10,000×g for 10 min at 4°C and take the supernatant to preserve it on ice for detection.

Tissue sample:

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 homogenate medium with a dounce homogenizer at 4°C.

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

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

Human serum: 8-10

Mouse serum: 8-10

10% Rat liver tissue homogenate: 2-3

10% Mouse brain tissue homogenate: 1

Human milk: 1

Human urine: 1

10% Mouse heart tissue homogenate: 1

10% Fish tissue homogenate: 1

Note: The diluent is homogenate medium. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. When washing the precipitate with anhydrous ethanol-ethyl acetate mixture application solution, the vortex must be sufficient. The mixing time should not be less than 1 min and the precipitate must be washed to white. If the precipitate still appears yellow, increase the washing times with anhydrous ethanol-ethyl acetate mixture application solution to ensure the washing process is sufficient. Otherwise the result will be higher.
2. The speed of centrifugation should not be reduced, otherwise the result will be higher.
3. It is recommended that round bottom test tubes instead of tip bottom tubes should be used to ensure full washing of the precipitate.
4. The protein content of the samples should range from 1-10 mg/mL.
5. Don't discard the supernatant, it needs to be used to determine the protein content after detecting the sample.

6. The protein content of the samples cannot be determined using the Bradford method.

9. Operating steps

1. **Sample pretreatment:** Serum (plasma), hydrothorax, cell supernatant: Detect the sample directly. Tissue sample: Take 0.45 mL the supernatant and add 0.05 mL of sulfates application solution. Stand for 10 min at room temperature, centrifuge at 11,580×g for 10 min at 4°C and take the supernatant for detection.
2. **The measurement of samples:** Sample tube: Add 0.1 mL of sample, 0.4 mL of DNPH solution into 2 mL EP tubes. Control tube: Add 0.1 mL of sample, 0.4 mL of acid reagent into 2 mL EP tubes.
3. Mix fully by swirling for 1 min and incubate for 30 min at 37°C with shading light.
4. Add 0.5 mL of protein precipitator, mix fully by swirling for 1 min, centrifuge at 13,780×g for 10 min at 4°C, discard the supernatant and keep the precipitate.
5. Add 1 mL of anhydrous ethanol-ethyl acetate mixture application solution, mix fully by swirling for 1 min, centrifuge at 13,780×g for 10 min at 4°C, discard the supernatant and keep the precipitate.
6. Repeat step 4 for 3 times (If the precipitate still appears yellow, increase the washing times with anhydrous ethanol-ethyl acetate mixture application solution to ensure the washing process is sufficient).
7. Add 1.25 mL of denaturant, mix fully by swirling and incubate at 37°C water bath for 15 min accurately.
8. Mix fully by swirling to dissolve the precipitate completely. Centrifuge at 13,780×g for 15 min at 4°C.
9. Take 300 μL of supernatant into the wells, and measure the OD values at 370 nm with reader. Meanwhile, determine the protein concentration of supernatant (Don't use the Bradford method to detect the protein concentration, MAES0177 and MAES0401 are recommended).

10. Calculation

The sample:

Protein carbonyl content (nmol/mgprot) = A_1-A_2 / (ε×d) ÷ (C_{pr} × V_1/V_2) × 10^6 × f

= (A_1-A_2) × 4.55 ÷ C_{pr} × f

[Note]

A_1: the OD value of sample.

A_2: the OD value of control.

ε: the molar extinction coefficient of carbonyl, 22,000 L/mol/cm.

d: the optical path of cuvette, 0.8 cm.

V_1: the total volume of reaction system, 1.25 mL.

V_2: the volume of sample added to the reaction system, 0.1 mL.

C_{pr}: the protein concentration of the sample supernatant, mgprot/mL.

10^6: unit conversion, 1 mol/L=10^6 nmol/mL.

f: dilution factor of sample before tested.

4.55: the constant after the formula simplification.

11. Appendix

Example Analysis

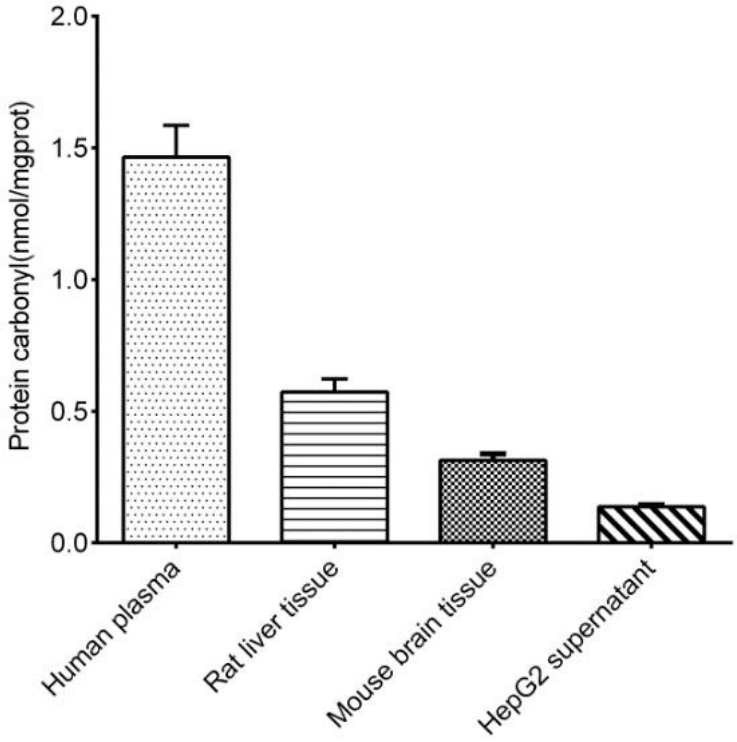
Example analysis:

Dilute the human plasma with double distilled water at a ratio of 1:9, take 0.1 mL of human plasma and carry out the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.082, the average OD value of the control is 0.069, the concentration of protein in sample supernatant is 0.43 mgprot/mL, and the calculation result is:

Protein carbonyl content (nmol/mgprot) = (0.082 - 0.069) × 4.55÷ 0.43× 10 = 1.38 nmol/mgprot

Detect human plasma (diluted 10 times, the concentration of protein in sample is 0.43 mgprot/mL), 10% rat liver tissue homogenate (diluted 2 times, the concentration of protein in sample is 0.29 mgprot/mL), 10% mouse brain tissue homogenate (the concentration of protein in sample is 0.23 mgprot/mL) and HepG2 supernatant (the concentration of protein in sample is 0.35 mgprot/mL) according to the protocol, the result is as follows:



12. 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 falls outside 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.

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