Technical Manual

Protein Carbonyl Colorimetric Assay Kit (Tissue And Serum Samples)

- Catalogue Code: MAES0097
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

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

Instructions should be followed strictly, 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

The reactive oxygen species produced by aerobic metabolism in the body can cause the oxidation of DNA, lipids and proteins. The secondary reaction of the amino acid side chain of the protein with the lipid oxidation product is the main cause of the formation of the carbonyl. Carbonyl is a biological marker of ROS-mediated protein oxidation.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Homogenate Medium	50 mL× 2 vials	2-8°C, 6 months
Sulfates	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
DNPH Solution	20 mL× 1 vial	2-8°C, 6 months, avoid direct sunlight
Acid Reagent	20 mL× 1 vial	2-8°C, 6 months
Protein Precipitator	60 mL× 1 vial	2-8°C, 6 months
Denaturant	50 mL× 3 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Water bath
- Centrifuge
- Microplate Reader (360-385 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Anhydrous ethanol
- Ethyl acetate

6. Assay Notes

1. When washing the precipitate with anhydrous ethanol-ethyl acetate mixture application solution, vortex thoroughly. The mixing time should not be less than 1 min and the precipitate must be washed until it becomes a white colour. If the precipitate still appears yellow, increase the washing time.

2. The speed of centrifuge should not be reduced.

3. It is recommended to use a round bottom test tube instead of the tip bottom tube to ensure that the precipitate is thoroughly washed.

4. The protein content of the samples can't be determined using the Bradford method.

5. The protein content of the samples should be ranged from 1-10 mg/mL.

7. Reagent Preparation

1. Bring all reagents to room temperature before use.

2. The preparation of **sulfates application solution:** Dissolve a vial of sulfates with 3 mL double distilled water and store at 2-8°C away from direct sunlight for 3 days.

3. The preparation of **anhydrous ethanol-ethyl acetate mixture application solution:** Mix anhydrous ethanol and ethyl acetate mixture at a ratio of 1:1. Prepare fresh solution before use.

8. Sample Preparation

1. Serum sample:
Collect fresh blood and place at 25°C for 30 min to clot the blood. Centrifuge the sample at 4°C for 15 min at 2000 g, take the upper yellowish clear liquid as serum. Place the serum on ice before detection.

2. Plasma sample:
Add the fresh blood into a test tube containing anticoagulant (heparin is recommended) and mix upside down. Centrifuge the sample at 4°C for 10 min at 700~1000 g, take the upper yellowish transparent liquid as the plasma. Place the plasma 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 excess water and weigh. Homogenize at the ratio of the volume of homogenate medium (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 it 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.

4. Hydrothorax sample:
Collect fresh hydrothorax sample into the tube which has anticoagulant, centrifuge at 10000 g for 10 min at 4°C. Take supernatant and preserve it on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

5. Cell culture supernatant:
Collect the fresh cell culture supernatant, centrifuge at 10000 g for 10 min at 4°C. Take the supernatant and preserve it on ice before detection.

Dilution of Samples:

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and the sample can be detected by this kit when the protein content of samples is ranged from 1-10 mg/mL.

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

Sample Type:	Dilution Factor
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 double distilled water or homogenate medium.

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	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

Note: S1-S48, sample wells; S1'- S48', control wells.

10. Operation Steps

Sample pre-treatment

1. Serum (plasma), hydrothorax, cell supernatant: Detect the sample directly.

2. Tissue sample: Take 0.45 mL of the supernatant and add 0.05 mL of sulfates application solution. Stand for 10 min at room temperature, centrifuge at 11580 g for 10 min at 4°C and take the supernatant for detection.

The Measurement of Sample:

1. **Sample tube:** Add 0.1 mL of sample and 0.4 mL of DNPH solution into 2 mL EP tubes.
   Control tube: Add 0.1 mL of sample and 0.4 mL of acid reagent into 2 mL EP tubes.

2. Mix fully by swirling for 1 min and incubate for 30 min at 37°C avoiding direct sunlight.

3. Add 0.5 mL of protein precipitator, mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4°C, discard the supernatant and keep the precipitate.

4. Add 1 mL of anhydrous ethanol-ethyl acetate mixture application solution, mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4°C, discard the supernatant and keep the precipitate.

5. Repeat step 4 three times (If the precipitate is still yellow, increase the washing times of anhydrous ethanol-ethyl acetate mixture application solution to ensure the washing process is sufficient).

6. Add 1.25 mL of denaturant, mix fully by swirling and incubate at 37°C for 15 min.

7. Mix fully by swirling to dissolve the precipitate. Centrifuge at 13780 g for 15 min at 4°C, then take the supernatant.

8. Add 300 μL of supernatant into the wells. Measure the OD values at 370 nm.

9. Determine the protein concentration of supernatant (MAES0177).

Operation Table

	Sample tube	Control tube
Sample (mL)	0.1	0.1
DNPH solution (mL)	0.4	
Acid reagent (mL)		0.4
Mix fully by swirling for 1 min, react away from direct sunlight at 37°C for 30 min.		
Protein precipitator (mL)	0.5	0.5
Mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4°C, discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4°C, discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4°C, discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4°C, discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4°C, discard the supernatant and keep the precipitate.		
Denaturant (mL)	1.25	1.25
Mix fully by swirling and incubate in a 37°C water bath for 15 min.		
Mix fully by swirling to dissolve the precipitate. Centrifuge at 13780 g for 15 min at 4°C Take 300 μL of the supernatant and measure the OD values at 370 nm with a microplate reader. Meanwhile, determine the protein concentration of supernatant (MAES0177).		

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11. Calculations

$$\text{Protein carbonyl content (nmol/mgprot)} = \frac{A1-A2}{\varepsilon \times d} \div \left(C_{pr} \times \frac{V1}{V2}\right) \times 10^6 \times f$$

$$= (A_1-A_2) \times 4.55 \div C_{pr} \times f$$

A_1: the OD value of sample.
A_2: the OD value of control.
ε: the molar extinction coefficient of carbonyl, 22000 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/L
10^6: unit conversion, 1 mol/L=106 nmol/mL
f: dilution factor of sample before tested
4.55: the constant after the formula simplification

12. Performance Characteristics

Average recovery rate (%)	97
Average inter-assay CV (%)	8.5
Average intra-assay CV (%)	5.2

Analysis

Dilute the human plasma with double distilled water at a ratio of 1:9, take 0.1 mL of human plasma and carry the assay according to the operation table.

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

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, please read the instructions carefully, and wear gloves and work clothes.

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