PRODUCT INFORMATION & MANUAL

Protein Carbonyls Assay Kit (Colorimetric)

NBP3-24531

For research use only.
Not for diagnostic or therapeutic procedures.

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Protein Carbonyls Assay Kit (Colorimetric)

Catalog No: NBP3-24531

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Instrument: Microplate reader

Average intra-assay CV (%): 5.2

Average inter-assay CV (%): 8.5

Average recovery rate (%): 97

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

The reactive oxygen species produced by the aerobic metabolism in the body can cause the oxidation of DNA, lipid and protein. 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.

▲Detection principle

The content of protein carbonyl increased after oxidation, and the carbonyl group reacted 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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Homogenate Medium	50 mL× 2 vials	2-8℃ , 12 months
Reagent 2	Sulfates	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	DNPH Solution	20 mL× 1 vial	2-8℃ , 12 months, shading light
Reagent 4	Acid Reagent	20 mL× 1 vial	2-8℃ , 12 months
Reagent 5	Protein Precipitator	60 mL× 1 vial	2-8℃ , 12 months
Reagent 6	Denaturant	50 mL× 3 vials	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (370 nm), Vortex mixer, Micropipettor, Water bath, Incubator, Centrifuge

Reagents:

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ 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 appear yellow, increase the washing times properly of anhydrous ethanol-ethyl acetate mixture application solution to ensure the washing process is sufficient. Otherwise the result will be higher.
2. The speed of centrifuge should not be reduced, otherwise the result will be higher.
3. It is recommended that the round bottom test tube instead of the tip bottom tube should be used to ensure fully washing of the precipitate.
4. The protein content of the samples should be ranged from 1-10 mg/mL.
5. Don't discard the supernatant, it needs to detect the protein content after detect the sample.
6. The protein content of the samples can't be determined using the Bradford method.

Pre-assay preparation

▲ Reagent preparation

1. The preparation of reagent 2 application solution

Dissolve a vial of reagent 2 with 3 mL double distilled water fully and it can be stored at 2-8℃ with shading light for 3 days.

2. The preparation of anhydrous ethanol-ethyl acetate mixture application solution

Mix anhydrous ethanol and ethyl acetate mixture at a ratio of 1:1. Prepared the fresh solution before use.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment. 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 reagent 1.

Assay protocol

▲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.

▲Detailed operation steps

1. Sample pretreatment

1) Serum (plasma), hydrothorax, cell supernatant: Detect the sample directly.
2) Tissue sample: Take 0.45 mL the supernatant and add 0.05 mL of reagent 2 application solution. Stand for 10 min at room temperature, centrifuge at 11580 g for 10 min at 4℃ and take the supernatant for detection.

2. The measurement of samples

1) Sample tube: Add 0.1 mL of sample, 0.4 mL of reagent 3 into 2 mL EP tubes.
   Control tube: Add 0.1 mL of sample, 0.4 mL of reagent 4 into 2 mL EP tubes.
2) Mix fully by swirling for 1 min and incubate for 30 min at 37℃ with shading light.
3) Add 0.5 mL of reagent 5, mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4℃ , 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℃ , discard the supernatant and keep the precipitate.
5) Repeat the step 4 for 3 times (If the precipitate still appear yellow, increase the washing times properly of anhydrous ethanol-ethyl acetate mixture application solution to ensure the washing process is sufficient).
6) Add 1.25 mL of reagent 6, mix fully by swirling and incubate at 37℃ water bath for 15 min accurately.
7) Mix fully by swirling to dissolve the precipitate fully. Centrifuge at 13780 g for 15 min at 4℃ .
8) Take 300 μL of supernatant into the wells, and measure the OD values at 370 nm with microplate reader. Meanwhile, determine the protein concentration of supernatant (Don't use the Bradford method to detect the protein concentration, are recommended).

▲Summary operation table

	Sample tube	Control tube
Sample (mL)	0.1	0.1
Reagent 3 (mL)	0.4	
Reagent 4 (mL)		0.4
Mix fully, react with shading light at 37℃ for 30 min accurately.		
Reagent 5 (mL)	0.5	0.5
Mix fully, centrifuge at 13780 g for 10 min at 4℃ , discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully, centrifuge at 13780 g for 10 min at 4℃ , discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully, centrifuge at 13780 g for 10 min at 4℃ , discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully, centrifuge at 13780 g for 10 min at 4℃ , discard the supernatant and keep the precipitate.		
Anhydrous ethanol-ethyl acetate mixture application solution (mL)	1.0	1.0
Mix fully, centrifuge at 13780 g for 10 min at 4℃ , discard the supernatant and keep the precipitate.		
Reagent 6 (mL)	1.25	1.25
Mix fully and incubate at 37℃ water bath for 15 min accurately.		
Mix fully. Centrifuge at 13780 g for 15 min at 4℃ , take 300 μL of supernatant into the wells, and measure the OD values at 370 nm. Meanwhile, determine the protein concentration of supernatant.		

▲Calculation

$$\text{Protein carbonyl content (nmol/mgprot)} = \frac{(A_1-A_2)}{(\varepsilon \times d)} \div (C_{pr} \times \frac{V_1}{V_2}) \times 10^6 \times f$$
$$= (A_1-A_2) \times 4.55 \div C_{pr} \times f$$

Note:

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=10^6 nmol/mL

4.55: the constant after the formula simplification

f: dilution factor of sample before tested.

Appendix I Data

▲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 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:

$$\text{Protein carbonyl content(nmol/mgprot)}=(0.082-0.069)\times 4.55\div 0.43\times 10$$

$$=1.38\ \text{nmol/mgprot}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

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

▲ Plasma

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

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenization medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Hydrothorax sample

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

▲ Cell culture supernatant

Collect the fresh cell culture supernatant, centrifuge at 10000 g for 10 min at 4℃ and take supernatant to preserve it on ice for detection.

Note:

1. Homogenized medium: reagent 1.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60, Hz 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)