Protein Carbonyl Colorimetric Assay Kit (Tissue and Serum Samples)

Introduction

Protein carbonylation refers to the process of oxidative modification of amino acid residues (such as lysine, arginine, threonine, proline, etc.) in protein molecules to form carbonyl (-C=O) groups, which is an important indicator to measure protein oxidative damage in vivo. The endogenous nature is mainly mediated by oxidative stress, such as reactive oxygen species (ROS) attacking amino acid side chains, Maillard reaction, or spontaneous oxidation of proteasome degradation intermediates, and can also be induced by the Fenton reaction catalyzed by metal ions (such as Fe^{2+}, Cu^{2+}). Exogenous is associated with environmental factors such as ultraviolet light, ionizing radiation, cigarette smoke, industrial contaminants (e.g., acrolein), and advanced glycation end products (AGEs) in the diet etc. In terms of disease association, the increase of protein carbonylation level is closely related to a variety of pathological processes, such as oxidative stress-related diseases (such as diabetes mellitus and cardiovascular diseases). Carbonylation can lead to protein structural destruction, abnormal function or aggregation, affecting enzyme activity, signal transduction and cytoskeletal stability. In neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease), aberrant carbonylated proteins (e.g., β-amyloid, α-synuclein) tend to form toxic aggregates and aggravate neuronal damage. In addition, processes such as cancer, chronic kidney disease, aging, etc., have also been implicated in imbalances of carbonylation modifications.

Protein carbonylation modification can be achieved by a variety of methods, the most classic and convenient of which is the reaction between 2,4-dinitrophenylhydrazine (DNPH) and protein carbonyl groups. The principle is that the hydrazine group ($-NHNH_2$) of DNPH undergoes a nucleophilic addition reaction with the carbonyl group (-C=O) in the protein to form a stable hydrazone derivative, which is analyzed spectrophotometrically at 370 nm.

This kit is designed to provide a simple and accurate method to quantify the carbonyl group in protein samples and is suitable for the detection of protein carbonyl content in serum, plasma, pleural fluid, cell supernatant, and tissue samples.

Components and Storage

Size / Components	48 T	96 T	Storage
Homogenate Medium	50 mL	2X 50 mL	4℃

Streptomycin sulfate	3X 1 vial	6X 1 vial	4°C protect from light
DNPH Solution	10 mL	20 mL	4°C protect from light
Acid Reagent	10 mL	20 mL	4℃
Protein Precipitator	30 mL	60 mL	4°C protect from light
Denaturant	2X 37.5 mL	3X 50 mL	4℃

Shipping: Blue ice **Shelf life: 6 months**

Protocol

1. Bring your own ingredients

   Microplate plate, microplate reader (360-385 nm, optimal detection wavelength 370 nm), double distilled water, absolute ethanol, ethyl acetate, propanol

2. Reagent preparation

   - Before testing, equilibrate the reagents in the kit to room temperature.
   - Preparation of Streptomycin sulfate working solution: 3 mL of double distilled water was added to 1 vial Streptomycin sulfate and mixed thoroughly, which was Streptomycin sulfate working solution. After configuration, the liquid can be stored at 2-8°C in the dark for 3 days.
   - Preparation of cleaning solution (anhydrous ethanol and ethyl acetate mixed application solution): mixed according to the ratio of absolute ethanol and ethyl acetate to 1:1, and it is ready to be used.
   - Propanol: Pre-cooled at -20°C in advance.

3. Sample preparation

   - Sample processing

   a. Cells: Cells (1~2X10^7) were collected, 2 mL of Homogenate Medium was added, and the supernatant was extracted by centrifugation at 11,580 × g for 10 min at 4°C after ultrasonic discion.

   b. Tissue: 2 mL of Homogenate Medium was added to 200 mg of tissue sample, followed by 50 uL of Streptomycin sulfate per 450 uL of Homogenate medium sulfate working solution, mix well, stand at room temperature for 5 min, and finally centrifuge at 4°C, 11,580 × g for 10 min to take the supernatant.

   c. Liquid samples such as plasma and serum: can be tested directly without complex pretreatment, but it is recommended to dilute according to the detection range of the kit.

***Note:** Different dilutions are recommended for samples tested for the first time to ensure the reliability of the experiment.

   - Sample dilution

The protein content of the sample is required to be between 1-10 mg/mL, and 2-3 samples with large, expected differences should be diluted into different concentrations for pre-experimentation before formal testing, and the

optimal dilution factor should be selected for formal batch experiment. The dilution ratios of different samples are listed on the table below (for reference only):

Sample type	Dilution factor
Serum (human/rat)	8-10 times
10% rat liver homogenate	2-3 times
10% mouse brain homogenate, 10% mouse heart homogenate, 10% fish meat homogenate, human milk, human urine	Not diluted
*Note: The diluent is double-distilled water or Homogenate Medium.	

4. Experimental manipulation

Follow the table below:

	Sample	Control
Sample	100 μL	100 μL
Acid Reagent	-	400 μL
DNPH Solution	400 μL	-
After vortexing and mixing, incubate at room temperature in the dark for 1 h, mixing once every 15 min		
Protein Precipitator	500 μL	500 μL
After vortexing and mixing, incubate on ice for 5 minutes, followed by centrifugation at 4°C at 12,000 rpm for 10 minutes, and discard the supernatant		
Ice acetone (bring your own, pre-cooled)	1 mL	1 mL
Pipette to stir up the pellet as much as possible; Subsequently, ultrasonic crushing was carried out for 30 s-1 min until the bulk precipitate was completely visible. Leave at -20°C for 5 min, then centrifuge at 4°C at 12,000 rpm for 4 min, and discard the supernatant		
Ice acetone (bring your own, pre-cooled)	1 mL	1 mL
Pipette to stir up the pellet as much as possible; Subsequently, ultrasonic crushing was carried out for 30 s-1 min until the bulk precipitate was completely visible. Leave at -20°C for 5 min, then centrifuge at 4°C at 12,000 rpm for 4 min, and discard the supernatant		
Cleaning solution (bring your own)	1 mL	1 mL
The Vortex was shaken for 1 minute, followed by centrifugation at 4°C at 12,000 rpm for 4 minutes, and the supernatant was discarded		
Cleaning solution (bring your own)	1 mL	1 mL
Vortex for 1 min, centrifuge at 4℃ and 12000 rpm for 4 min, and discard the supernatant.		
If there is still a yellow residue in the sediment, increase the cleaning times of the cleaning solution until the precipitation is colorless		

Denaturing	1.25 mL	1.25 mL
Vortex for 1 min and then incubate at 37℃ for 15 min.		

After centrifugation at 4°C and 12000 rpm for 15 min, the pellet was discarded (most of them had no precipitation), and the supernatant was transferred to a new EP tube. Use a micropipette to take an appropriate amount of supernatant to the microplate plate (for example, add 300 μL of supernatant to a 96-well plate), and the OD value at 370 nm was determined by the microplate reader. At the same time, the supernatant was taken, and the protein concentration was determined using the BCA method (Cat. No. K4101).

***Note:** For the BCA assay (K4101), the BSA in the kit needs to be diluted with Denaturant.

5. Result calculation

The carbonyl content：

$$\frac{A_1 - A_2}{\varepsilon \times d} \div \left(C_{\mathrm{pr}} \times \frac{V_1}{V_2} \right) \times 10^6 \times f = (A_1 - A_2) \times 3.64 \div C_{\mathrm{pr}} \times f$$

***Note:**

A1: OD value of the sample group

A2: OD value of the control group

ε: Carbonyl molar extinction coefficient, 22000 L/mol/cm

d: Colorimetric light path (1 cm)

V1: Total reaction volume (1.25 mL)

V2: Add to the reaction sample volume to be measured (0.1 mL))

CPR: Determine the protein concentration of the tube supernatant (mgprot/mL)

106: unit conversion, 1 mol/L=106 nmol/mL

f: The dilution of the sample before it was added to the assay system

3.64: constant

Example: Mouse serum dilution was 8-fold, the measured protein concentration was 0.51 mg/mL, the OD value of the sample group was 0.027, and the OD value of the control group was 0.021, and the calculated results were:

The carbonyl content =（0.027-0.021）x 3.64÷0.51 x 8=0.347 nmol/mgprot

Note

1. The protein content of the sample to be tested ranged from 1-10 mg/mL.
2. The supernatant cannot be measured by the Coomassie Brilliant Blue method, and the BCA method is recommended.
3. When pipetting the supernatant onto the plate, avoid air bubbles.
4. Animal tissue samples are washed of surface blood with PBS (0.01 M, pH 7.4).
5. This product is for scientific research use only.

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