Protein Carbonyl assay kit

(Cat/No.:BC117 Size:100T/48S,50T/24S)

1. Composition and preparation: (Kit Shelf life: 3 months)

Reagent composition	Reagent status	50T / 24S	100T / 48S	Storage conditions
Reagent 1	Homogenization medium	50mL×1 bottle	100mL×1 bottle	4℃
Reagent 2	Light brick-red liquid	2.5mL×1 bottle	5mL×1 bottle	4 °C, protected from light
Reagent 3	Yellow liquid	10mL×1 bottle	20mL×1 bottle	4 °C, protected from light
Reagent 4	Colorless liquid	10mL×1 bottle	20mL×1 bottle	4℃
Reagent 5	Colorless liquid	30mL×1 bottle	60mL×1 bottle	4℃
Reagent 6	Colorless liquid	70mL×1 bottle	70mL×2 bottle	4℃

2. Required instruments and reagents

UV spectrophotometer and quartz cuvettes, vortex mixer, high-speed centrifuge (12000 rpm), absolute ethanol (analytical grade), ethyl acetate (analytical grade), protein assay reagents.

3. Operation procedure

1. Serum (plasma) samples: Directly take samples for determination;

2. Pretreatment of tissue samples: Accurately weigh the tissue sample, add 9 volumes of Reagent 1 according to the ratio of weight (g): volume (mL) = 1:9 (e.g., accurately weigh 0.1 g tissue and add 0.9 mL Reagent 1), mechanically homogenize in an ice-water bath, centrifuge at 2500 rpm for 10 min, take the supernatant, 450 μL of 10% homogenate, add 50 μL Reagent 2, mix well, incubate at room temperature for 10 min, then centrifuge at 11000 r/min for 10 min, and take the supernatant for determination according to the operation table;

3. Operation table:

	Determination tube	Control tube
Sample (mL)	0.1	0.1
Reagent 3 (mL)	0.4	
Reagent 4 (mL)		0.4

Vortex mix for 1 minute, react at 37 °C protected from light for 30 minutes		
Reagent 5 (mL)	0.5	0.5
First wash: vortex mix for 1 minute, centrifuge at 12000 r/min for 10 min at 4 °C, discard the supernatant and retain the precipitate		
Wash solution (mL)	1.0	1.0
Second wash: vortex mix for 1 minute, centrifuge at 12000 r/min for 10 min at 4 °C, discard the supernatant and retain the precipitate		
Wash solution (mL)	1.0	1.0
Third wash: vortex mix for 1 minute, centrifuge at 12000 r/min for 10 min at 4 °C, discard the supernatant and retain the precipitate		
Wash solution (mL)	1.0	1.0
Fourth wash: vortex mix for 1 minute, centrifuge at 12000 r/min for 10 min at 4 °C, discard the supernatant and retain the precipitate		
Wash solution (mL)	1.0	1.0
Fifth wash: vortex mix for 1 minute, centrifuge at 12000 r/min for 10 min at 4 °C, discard the supernatant and retain the precipitate		
Reagent 6 (mL)	1.25	1.25
After mixing, incubate in a water bath at 37 °C for 15 minutes. Remove, vortex mix, centrifuge at 12000 r/min for 15 min, take the supernatant, and measure the OD value of each tube at 370 nm (ultraviolet) using a 0.5 cm path-length quartz cuvette, with Reagent 6 as the blank.		

4. Calculation formula

$$\text{Protein carbonyl content (nmol/mgprot)} = \frac{A_{\text{Determination}} - A_{\text{Control}}}{22 \times d \times Cpr} \times 125 \times 10^5$$

d: optical path length, cm;
Cpr: protein concentration of the sample, mgprot/L (prot refers to protein).

5. Calculation examples

Example 1: Take 0.1 mL heparin-anticoagulated human plasma and operate according to the instructions. The absorbance of the control tube was measured as 0.006, and the absorbance of the determination tube was 0.036. The total protein concentration of human plasma measured using a biuret kit was 54.3 mgprot/mL. Substitute the data into the calculation formula:

$$\text{Protein carbonyl content (nmol/mgprot)} = \frac{0.036 - 0.006}{22 \times 0.5 \times 54.3 \times 1000} \times 125 \times 10^5$$

$$= 0.628\ \text{nmol/mgprot}$$

Example 2: Take rat lung tissue, prepare a 10% homogenate with Reagent 1, and operate according to the instructions. The absorbance of the control tube was measured as

0.012, and the absorbance of the determination tube was 0.026. The total protein concentration of the supernatant was 6.52 mgprot/mL. Substitute the data into the calculation formula:

$$\text{Protein carbonyl content (nmol/mgprot)} = \frac{0.026 - 0.012}{22 \times 0.5 \times 6.52 \times 1000} \times 125 \times 10^5$$
$$= 2.44\,\text{nmol/mgprot}$$

6. Assay significance

This kit is used for the determination of protein carbonyl content. It allows convenient and sensitive detection of protein carbonyls in various tissues and organs, serum, cultured cells (processed in the same way as tissue samples), and organelles. The assay does not require expensive equipment, and sample preparation is simple. It can be widely applied to the early diagnosis of diseases such as aging, atherosclerosis, diabetes, Parkinson's syndrome, and rheumatoid arthritis, as well as to the evaluation of antioxidant health products, antioxidant drugs, and cosmetics. In addition, it can be used to evaluate oxidative damage to organisms caused by environmental harmful factors such as radiation and chemical toxins.

7. Experimental notes

1. When washing the reaction precipitate with the mixed solution of absolute ethanol and ethyl acetate, vortex mixing must be vigorous and sufficient, and the mixing time should not be less than one minute. The precipitate must be washed until it becomes white. If yellow residue remains, appropriately increase the number of washing steps with the mixed solution of absolute ethanol and ethyl acetate to ensure thorough washing; otherwise, the measurement results may be falsely elevated;
2. During protein carbonyl determination, the centrifugation speed should not be arbitrarily reduced; otherwise, the measurement results may be falsely elevated;
3. During protein carbonyl determination, it is recommended to use round-bottom centrifuge tubes to ensure thorough washing of the precipitate during washing with the mixed solution of absolute ethanol and ethyl acetate. The use of conical-bottom centrifuge tubes is not recommended.