Protein Carbonyl Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ microplate reader

Cat No: BC1275

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Powder×5	2-8℃
Reagent II	Liquid 8 mL×1	2-8℃
Reagent III	Liquid 8 mL×1	2-8℃
Reagent IV	Liquid 18 mL×1	2-8℃
Reagent V	Requird but not provided	-
Reagent VI	Liquid 25 mL×1	2-8℃

Solution Preparation:

1. Reagent I: Before use, according to the number of samples, each branch is dissolved in 1 mL water, each branch is 10 sample dosage.
2. Reagent V: Self provided. ((Ethyl acetate and absolute ethanol are mixed in equal volume (1:1) according to the amount of sample.)

Product Description:

Protein carbonyl group is an early sign of various amino acids in the process of oxidative modification of proteins. The carbonyl content of protein can indicate the degree of oxidative damage of protein, and it is the main index to measure the oxidative damage of protein.

Carbonyl group can react with 2,4-dinitrophenylhydrazine to form red 2,4-dinitrophenylhydrazone with characteristic absorption peak at 370 nm.

Required material:

Balance, constant temperature water bath, low temperature centrifuge, vortex mixer, Spectrophotometer/ microplate reader, micro quartz cuvette/96-well flat-bottom plate (UV), distilled water, anhydrous ethanol, ethyl acetate.

Procedure:

I. Sample Extraction:

Tissue samples: Add 1 mL of Extract solution to 0.1 g of tissue sample, After full homogenization, centrifuge at 4℃ and 5000 rpm for 10 min. Take the supernatant. Add 0.1 mL of Reagent I. Place it at room temperature for 10 min and centrifuge at 4℃ and 12000 rpm for 10 min. Take the supernatant.

The protein content is then measured for 20 μL and the rest is used as samples to be tested.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 min, adjust the wavelength to 370 nm and set zero with Reagent VI.
2. Operation table:

Reagent name (μL)	Blank tube(B)	Test tube(T)
Sample	60	60
Reagent II		120
Reagent III	120	
Mix thoroughly; React at 37℃ for 1 h in shadow.		
Reagent IV	150	150
Stand for 5 min, and then centrifuge at 12000 rpm and 4℃ for 15 min, discard supernatant, left precipitation for use.		
Reagent V	300	300
Vortex fully, centrifuge at 12000 rpm and 4℃ for 10 min, discard supernatant, left precipitation for use, repeat for three times.		
Reagent VI	300	300
Vortex fully， and then incubate at 37℃ for 15 min, After the precipitate has dissolved completely, centrifuge at 12000 rpm and 4℃ for 15 min, Take 200 μL of supernatant into quartz cuvette or 96-well flat-bottom plates (UV), set zero with Reagent VI, measured the absorbance of 370 nm.		

III. Calculation:

a) Micro quartz cuvette

1. Calculated by sample protein concentration:

Protein Carbonyl（μmol/mg prot）=（$OD_{370\ test}$－$OD_{370\ blank}$）÷（ε×d）×V_{RVI}÷(Cpr×V_S)

=（$OD_{370\ test}$－$OD_{370\ blank}$）÷4.4÷Cpr;

2. Calculated by sample fresh weight:

Protein Carbonyl（μmol/g）=（$OD_{370\ test}$－$OD_{370\ blank}$）÷（ε×d）×V÷(W×V_S÷Ve)

=（$OD_{370\ test}$－$OD_{370\ blank}$）÷4÷W

ε: Protein carbonyl extinction coefficient, 22 mL• μmol^{-1}•cm^{-1};

d: the optical diameter of cuvette, 1 cm;

V_{RVI}: The volume of added Reagent VI, 0.3 mL;

V_S: Add sample volume, 0.06 mL； ;

Ve: Add volume of Extract solution and Reagent I, 1.1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g.

b) 96-well flat-bottom UV plate

Replace d-1cm (the optical diameter of cuvette) to d-0.6cm (the optical diameter of 96-well flat-bottom plate) for calculation.

Note:

1. The reagent is ready-mixed according to the number of samples to be determined before use. It is stored at 4℃. If it turns black, it cannot be used.
2. Reagent II is easy to decompose at light, so the reaction should be strictly avoided from light.

Examples:

1. Add 0.1g liver to 1mL extract solution and mix thoroughly, centrifuge with 5000rpm at 4℃ for 10min, take supernatant and add 0.1ml Reagent I at room temperature for 10min, centrifuge with 12000rpm at 4℃ for 10min, take supernatant, follow the determination procedure to operate, with micro quartz cuvette to calculate: $OD_{370test}$=0.1017, $OD_{370blank}$=0.0385, according with mass of sample to calculate Protein Carbonyl（μmol/g weight）=（$OD_{370test}$-$OD_{370blank}$）÷4÷W=0.158 μmol/g weight.

2.Add 0.1g purple flower to 1mL extract solution and mix thoroughly, centrifuge with 5000rpm at 4℃ for 10min, take supernatant and add 0.1ml Reagent I at room temperature for 10min, centrifuge with 12000rpm at 4℃ for 10min, take supernatant, follow the determination procedure to operate, with micro quartz cuvette to calculate: $OD_{370test}$=0.0347, $OD_{370blank}$=0.0022, according with mass of sample to calculate Protein Carbonyl（μmol/g weight）=（$OD_{370test}$-$OD_{370blank}$）÷4÷W=0.0875 μmol/g weight.

Related Products:

BC3590/BC3595	Hydrogen Peroxide (H2O2) Content Assay Kit
BC0020/BC0025	Malondialdehyde (MDA) Content Assay Kit
BC1090/BC1095	Xanthine Oxidase(XOD) Activity Assay Kit
BC0690/BC0695	Glucose Oxidase (GOD) Activity Assay Kit