Protein Carbonyl Content Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Cat No: AK0459

Size: 100T/48S

Components:

Extract solution: Liquid 50 mL×1. Storage at 4℃ .

Reagent I: powder 0.1 g×5. Storage at 4℃ . (Before use, according to the number of samples, each branch is dissolved in 1 mL of distilled water, each branch is 10 sample dosage.)

Reagent Ⅱ: Liquid 6 mL×1. Storage at 4℃ and protected from light.

Reagent Ⅲ: Liquid 6 mL×1. Storage at 4℃ .

Reagent Ⅳ: Liquid 15 mL×1. Storage at 4℃ .

Reagent Ⅴ: Self provided. (Ethyl acetate and absolute ethanol are mixed in equal volume (1:1) according to the amount of sample.)

Reagent Ⅵ: Liquid 30 mL×1. Storage at 4℃ .

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 4C 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 4C 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:

<table>
<tr><td>Reagent name (μL)</td><td>Blank tube(B)</td><td>Test tube(T)</td></tr>
<tr><td>Sample</td><td>60</td><td>60</td></tr>
<tr><td>Reagent Ⅱ</td><td></td><td>120</td></tr>
<tr><td>Reagent Ⅲ</td><td>120</td><td></td></tr>
<tr><td colspan="3">Mix thoroughly; React at 37C for 1 h in shadow.</td></tr>
<tr><td>Reagent Ⅳ</td><td>150</td><td>150</td></tr>
<tr><td colspan="3">Stand for 5 min, and then centrifuge at 12000 rpm and 4°C for 15 min, discard supernatant, left precipitation for use.</td></tr>
<tr><td>Reagent Ⅴ</td><td>300</td><td>300</td></tr>
<tr><td colspan="3">Vortex fully, centrifuge at 12000 rpm and 4°C for 10 min, discard supernatant, left precipitation for use, repeat for three times</td></tr>
<tr><td>Reagent Ⅵ</td><td>300</td><td>300</td></tr>
<tr><td colspan="3">Vortex fully， and then incubate at 37°C for 15 min, After the precipitate has dissolved completely, centrifuge at 12000 rpm and 4°C 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.</td></tr>
</table>

III. Calculation:

a) Micro quartz cuvette

1. Calculated by sample protein concentration:

Protein Carbonyl（μmol/mg prot）$= (OD_{370\,test} - OD_{370\,blank}) \div (\varepsilon \times d) \times V_{RVI} \div (Cpr \times V_S)$

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

2. Calculated by sample fresh weight:

Protein Carbonyl（μmol/g）$= (OD_{370\,test} - OD_{370\,blank}) \div (\varepsilon \times d) \times V \div (W \times V_S \div Ve)$

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

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°C . 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:

AK0362/AK0361	Hydrogen Peroxide (H2O2) Content Assay Kit
AK0662/AK0612	Malondialdehyde (MDA) Content Assay Kit
AK0490/AK0489	Xanthine Oxidase(XOD) Activity Assay Kit
AK0522/AK0521	Glucose Oxidase (GOD) Activity Assay Kit