Protein Carbonyl Content Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0772

Size: 50T/24S

Components:

Extract solution: Liquid 25 mL×1. Storage at 4℃.

Reagent I: powder 0.1 g×3, Storage at 4℃. (Before use, according to the number of samples, each branch is dissolved in 1 mL water, each branch is 10 sample dosage.)

Reagent II: Liquid 10 mL×1. Storage at 4℃ and protected from light.

Reagent III: Liquid 10 mL×1. Storage at 4℃.

Reagent IV: Liquid 25 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 50 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.

Reagents and Equipment Required but Not Provided:

Analysis balance, constant temperature water bath, centrifuge, vortex mixer, spectrophotometer, 1 mL quartz cuvette, distilled water, anhydrous ethanol and ethyl acetate.

Procedure:

I. Sample preparation：

Tissue samples: Add 1 mL 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 was then measured for 20 μL and the rest was used as samples to be tested.

II. Determination procedure:

1. Preheat the spectrophotometer for 30 min, adjust the wavelength to 370 nm and set zero with Reagent VI.

2. Add reagents with the following list:

Reagent name (mL)	Blank tube (B)	Test tube (T)

Sample	0.2	0.2
Reagent II		0.4
Reagent III	0.4	
Mix thoroughly; React at 37℃ for 1 h in shadow.		
Reagent IV	0.5	0.5
Stand still for 5 min; Centrifuge at 4℃, 12000 rpm for 10 min, discard supernatant and retain sediment.		
Reagent V	1.0	1.0
Vortex fully, Centrifuge at 4℃, 12000 rpm for 10 min, discard supernatant and retain the sediment. Repeat three times.		
Reagent VI	1.0	1.0
Vortex fully, and then incubate at 37℃ for 15 min,; After all the precipitates are dissolved, centrifuge at 4℃ and 12000 rpm for 15 min. Take the supernatant. Measure the absorbance of 370 nm with 1 mL quartz cuvette; Adjust to zero by Reagent VI.		

III. Calculation:

1. Calculated by sample protein concentration:

Protein Carbonyl（μmol/mg prot）=（$OD_{370\ test}$－$OD_{370\ blank}$）÷（ε×1）×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×1）×V÷(W×V_S÷Ve)

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

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

l: Optical path, 1 cm;

V_{RVI}: Added the volume of Reagent VI, 1 mL;

V_S: Add the volume of sample, 0.2 mL；;

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

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g.

Note:

1. The reagent should be 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. Reagents II is easy to decompose at sight, 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, and calculate:

$OD_{370test}$=0.102, $OD_{370blank}$=0.039, according with mass of sample to calculate Protein Carbonyl（μmol/g weight）=（$OD_{370test}$-$OD_{370blank}$）÷4÷W=0.1575 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, and calculate: $OD_{370test}$=0.037, $OD_{370blank}$=0.002, 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:

NA0789/NA0548 Xanthine Oxidase(XOD) Activity Assay Kit

NA0814/NA0572 Glucose Oxidase (GOD) Activity Assay Kit

NA0771/NA0530 Diamine oxidase(DAO) Activity Assay Kit