Ver. 241201

Protein Carbonyl Content Assay Kit

BC1270-1 (50 Tests/24 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

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 to protein, and it is the main index to measure the oxidative damage of protein.

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

Kit components

Reagent	Volume	Storage
Extraction Solution	25mL	2-8°C
Reagent I	20mL	2-8°C, in dark
Reagent II	10mL	2-8°C
Reagent III	40mL	2-8°C
Reagent IV	To be prepared by the user Ethyl acetate and absolute ethanol mixed in the ratio 1:1 according to the number of sample	
Reagent V	60mL	2-8°C

Reagents and Equipment Required but Not Provided

Centrifuge, Microplate reader/spectrophotometer, water bath, micro quartz cuvette/96 well UV flat-bottom plate, adjustable pipette and distilled water.

Sample Preparation

Tissue:

Add 1mL Extraction Reagent to 0.1g tissue. Homogenise the tissue completely while placed on ice. Centrifuge at 3000 ×g for 10 minutes at 4°C. Protein content is to measured from 20μL supernatant with BCA Protein Assay Kit (PC0020) and the rest is to be used for Protein carbonyl content assay.

Operation Procedures

1. Preheat the spectrophotometer reader for 30 minutes, adjust wave length to 370nm and set zero with Reagent IV.
2. Add reagents to 1mL glass cuvette as follows

Reagent	Blank (B)	Test (T)
Sample	-	0.2mL
Reagent I	-	0.4mL
Reagent II	0.4mL	-
Mix thoroughly. Incubate at 37°C for 1 hour in dark.		
Reagent III	0.5mL	0.5mL
Keep for 5 minutes without disturbing the mixture. Centrifuge at 12000 rpm, 4°C for 10 minutes. Discard supernatant and retain sediments.		
Reagent IV	1mL	1mL
Vortex, centrifuge 12000 rpm, 4°C for 10 min. Discard supernatant and retain sediments. Repeat 3 times		
Reagent V	1mL	1mL

3. Vortex vigorously and incubate at 37°C for 15 minutes.
4. After all the precipitate is dissolved, centrifuge at 12000 rpm, 4°C for 15 minutes.
5. Measure absorbance of the supernatant at 370 nm in 1 mL quartz cuvette.
6. Record A_B and A_T for Blank and Test respectively.

Calculations

1. ***Protein concentration:***
   Protein carbonyl (µmol/mg Prot) = $(A_T - A_B) \div 4.4 \div Cpr$

2. ***Sample weight:***
   Sialic Acid (SA) Content (mmol/g weight) = $(A_T - A_B) \div 4.4 \div W$

   Cpr : Sample protein concentration (mg/mL)
   W : Sample weight in grams.

Notes

- Reagent should be ready-mixed according to the number of samples to be determined.
- If reagents turn black, discard and do not use it.
- Reagent II is light sensitive. The reaction should be carried out in dark.