Protein Carbonyl Assay Kit

Cat#: orb1499782 (manual)

Size: 50T/48S

Visible Spectrophotometer

Product composition and storage conditions:

No.	Specifications	Storage Conditions
Extraction solution ES35	50 ml ×1	4 °C
orb1499782- A	Powder ×5	4°C, protected from light. (Prior to use, add 1 mL of distilled water to each vial according to the number of samples and use 10 samples per vial)
orb1499782- B	20mL×1	4°C, protected from light.
orb1499782- C	20ml×1	4 °C
orb1499782- D	25ml×1	4 °C
orb1499782- E	Self-provided	Ethyl acetate and anhydrous ethanol are mixed in equal volumes according to the determined sample volume.
orb1499782- F	100ml×1	4 °C

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

Introduction:

Significance: Oxidative stress occurs when the effectiveness of antioxidant defenses is insufficient to handle the production of reactive oxygen species (ROS). ROS can cause damage to DNA, lipids, and proteins. Oxidation of proteins produces stable carbonyl groups, which are early markers of oxidative modification of proteins and can be used as a means of detecting oxidative damage. Its content indicates the extent of oxidative damage of protein, which is the main indicator of oxidative damage of protein.

Principle: Protein carbonyl content determination testing kit provides a simple and direct method for the determination of carbonyl content in various biological samples. The carbonyl content is determined by derivatizing the protein carbonyl with 2,4-dinitrophenylhydrazine (DNPH) to form a stable dinitrophenylhydrazine (DNP) hydrazone adduct, which can be detected spectrophotometrically at 370nm in proportion to the carbonyl group present.

Own supplies:

Visible spectrophotometer, centrifuge, adjustable pipette , 1 ml glass cutlery, 100% ethanol, ethyl acetate.

Sample processing:

1. Tissue：According to the ratio of tissue weight (g): Extraction solution ES35 volume (mL): 1:5 ~ 10 (about 0.1 g tissue should be taken and 1 mL Extraction solution ES35 should be added) , homogenized in ice bath, centrifuged at 4000 g 4°C for 10 min, take the supernatant. Then add 0.1mL orb1499782 -A, place at room temperature for 10 min, centrifuge at 4°C10000 g for 10 min, and take supernatant for test.

2. Bacteria or fungi: According to the number of bacteria (10^4), Extraction solution ES35 volume (mL) is 500 ~ 1000:1 ratio (5 million bacteria are recommended to add 1 mL Extraction solution ES35) , the bacteria were broken by ultrasonic wave (ice bath, power 300 W, ultrasonic 3 s, interval 7 s, total time 3min) ; Then centrifuge for 10 min at 10000 g, 4°C, and take the supernatant and place it on ice for testing.

3. Liquid sample (body fluids or culture fluids:): use and test directly.

Measurement steps:

1. Preheat the visible spectrophotometer for at least 30 minutes, adjust the wavelength to 370nm, zero adjustment with orb1499782-F.

2. Add the following reagents in sequence to the EP tube:

Reagent name	Control tube (μl)	Measuring tube (μl)
Sample	200	200
orb1499782-B		400
orb1499782-C	400	
Mix well and react in 37°C for 1h without light		
orb1499782- D	500	500
Allow to stand for 5 minutes, centrifuge for 15 min at 4° C ,12000 rpm, discard the supernatant and leave the precipitate.		
orb1499782- E	1000	1000
Vortex and mix well, centrifuge for 10 min at 4° C , 12000 rpm, discard supernatant, and leave precipitate.		
orb1499782- E	1000	1000
Vortex and mix well, centrifuge for 10 min at 4° C , 12000 rpm, discard supernatant, and leave precipitate.		
orb1499782- E	1000	1000
Vortex and mix well, centrifuge for 10 min at 4° C , 12000 rpm, discard supernatant, and leave precipitate.		
orb1499782- F	1000	1000
Vortex and mix well, incubate for 15 min with 37° C, dissolve all precipitates, centrifuge for 15 min at 4° C , 12000 rpm, take 1000 μL supernatant in the cuvette, adjust to zero with orb1499782 -F, measure the absorbance of control tube and measuring tube at 370nm, and record $A_{measuring}$ and $A_{control}$ respectively. $\Delta A = A_{measuring} - A_{control}$.		

Note: control tubes only need to be done once.

Protein Carbonyl content calculation:

1. Calculated by sample protein concentration

Protein Carbonyl content (μmol/mg prot) = [ΔA ×V_{total} ÷ (ε×d)]÷ (V_{sample} ×Cpr)= 0.227×ΔA ÷Cpr

2. Calculated by sample weight

Protein Carbonyl content (μmol/g) = [ΔA ×V_{total} ÷ (ε×d)]÷ (W×V_{sample} ÷$V_{sample\ total}$) = 0.227×ΔA ÷W

3. Calculated by number of cells

Protein Carbonyl content (μmol /10^4 cell) = [ΔA ×V_{total} ÷(ε×d)]÷ (Number of cells ×V_{sample} ÷$V_{sample\ total}$) = 0.227×ΔA ÷ number of cells

4. Calculated by liquid volume

Protein Carbonyl content (μmol/mL) = [ΔA ×V_{total} ÷(ε×d)]÷V_{sample} = 0.227×ΔA

V_{total}: Total volume of reaction system, 1mL; ε: Micromolar extinction coefficient of carbonyl group, 22×10^{-3} L/μmol/cm; d: Cuvette light path, 1 cm; V_{sample}: Sample volume added, 0.2 mL; $V_{sample\ total}$: Volume of extract added, 1 mL; Cpr: Sample protein concentration, mg/mL, W: Sample weight, g;

Precautions:

1. orb1499782 -A is prepared according to the number of samples to be determined before use, and stored in 4°C after configuration. If it turns black, it cannot be used.
2. orb1499782 -B breaks down easily under light, so the reaction needs to be strictly protected from light.
3. The reagent in this kit contains organic solvent. Please wear disposable gloves and masks for protection.