Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Protein Carbonyl Assay Kit

Cat #: KTB1200 Size: 48 T/96 T

	Micro Protein Carbonyl Assay Kit		
REF	**Cat #:** KTB1200	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Protein carbonyl is an early sign of a variety of amino acids in the protein oxidative modification process, and its content indicates the protein oxidative damage degree, which is the main indicator to measure protein oxidative damage. The principle is carbonyl group reacts with 2,4-dinitrophenylhydrazine to produce red 2,4-dinitrophenylhydrazone with a characteristic absorption peak at 370 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Antioxidant	1	1	4℃, protected from light
Chromogen	6 mL	12 mL	4℃, protected from light
HCl	6 mL	12 mL	4℃
TCA	15 mL	30 mL	4℃
Guanidine Hydrochloride	30 mL	60 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 370 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water, ethyl alcohol, ethyl acetate
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Working Antioxidant: Prepare according to the sample number, take 0.1 g and dissolve it with 1 mL deionized water, 1 mL can be used for 10 samples. Store at 4℃, protected from light.
Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
HCl: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
TCA: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Guanidine Hydrochloride: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

1. Animal and Plant Tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 4,000 g for 10 min at 4°C. Take the supernatant, add 0.1 mL Working Antioxidant, keep at room temperature for 10 min, centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
3. Serum (Plasma) sample: Tested directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 370 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Operate according to the sample addition and reaction process in the following table:

Reagent	Control Tube (μL)	Test Tube (μL)
Sample	60	60
Chromogen	0	120
HCl	120	0
Mix well, 37℃, react in darkness for 1 h		
TCA	150	150
Keep it still for 5 min. 4℃, 12,000 g, centrifuge for 15 min, discard supernatant and keep the precipitation		
Ethyl Alcohol	150	150
Ethyl Acetate	150	150
Mix by vortex, 4℃, 12,000 g centrifuge for 10 min, discard supernatant and keep the precipitation, repeat 3 times		
Guanidine Hydrochloride	300	300
Mix by vortex, incubate at 37℃ for 15 min till the precipitate was completely dissolved. 4℃, 12,000 g, centrifuge for 15 min. Take 200 μL supernatant and add into 96-well plate or microglass cuvette, record the absorbance at 370 nm. Calculate $\Delta A=A_{Test}-A_{Control}$.		

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

1. Calculated by protein concentration

Protein carbonyl content (μmol/mg prot)=[ΔA×V_{Total}÷(ε×d)]÷(V_{Sample}×Cpr)**=0.454×ΔA÷Cpr**

2. Calculated by sample weight

Protein carbonyl content (μmol/g fresh weight)=[ΔA×V_{Total}÷(ε×d)]÷(W×V_{Sample}÷$V_{Total\ Sample}$)**=0.454×ΔA÷W**

3. Calculated by cells or bacteria number

Protein carbonyl content (μmol/10^4)=[ΔA×V_{Total}÷(ε×d)]÷(500×V_{Sample}÷$V_{Total\ Sample}$)**=0.454×ΔA÷500**

4. Calculated by liquid volume

Protein carbonyl content (μmol/mL)=[ΔA×V_{Total}÷(ε×d)]÷V_{Sample}**=0.454×ΔA**

Where: ΔA=A_{Test}-$A_{Control}$; V_{Total}: Total reaction volume, 0.3 mL; ε: Carbonyl molar extinction coefficient, 22 L/mmol/cm; d: 96-well plate diameter, 0.5 cm; V_{Sample}: Sample volume added, 0.06 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight; g; 500: Total number of cells or bacteria, 5×10^6.

B. Microglass cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Note: If the calculation method based on sample protein concentration, it is recommended to use Abbkine Protein Quantification Kit (BCA Assay) (Cat #:KTD3001).

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Assay Kit
KTB1080	CheKine™ Micro Superoxide Anion Scavenging Capacity Assay Kit
KTB1090	CheKine™ Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.