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/48 S 96 T/96 S

	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 6 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	60 mL	120 mL	4℃
Antioxidant	Powder×1 vial	Powder×1 vial	4℃, protected from light
Chromogen	7.5 mL	15 mL	4℃, protected from light
HCl	7.5 mL	15 mL	4℃
TCA	18 mL	36 mL	4℃
Guanidine Hydrochloride	36 mL	72 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 370 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well UV plate or micro quartz 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. Working Antioxidant is freshly prepared.
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℃.
Note: Antioxidant or Chromogen is toxic, TCA and Guanidine Hydrochloride has a pungent odor, so it is recommended to experiment in a fume hood.

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℃. 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℃. 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℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum (Plasma) sample: Tested directly.

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

Assay Procedure

1. Preheat the microplate reader or ultraviolet 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 UV microplate or micro quartz cuvette, record the absorbance at 370 nm.		

Calculate $\Delta A=A_{Test}-A_{Control}$.

Note: Each Test Tube should be provided with a Control Tube. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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 UV plate calculation formula

1. Calculated by protein concentration

Protein carbonyl content (μmol/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)]\div(V_{Sample}\times Cpr)$**$=0.454\times\Delta A\div Cpr$**

2. Calculated by sample weight

Protein carbonyl content (μmol/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)]\div(W\times V_{Sample}\div V_{Total\ Sample})$**$=0.454\times\Delta A\div W$**

3. Calculated by cells or bacteria number

Protein carbonyl content (μmol/10^4)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)]\div(500\times V_{Sample}\div V_{Total\ Sample})$**$=0.454\times\Delta A\div 500$**

4. Calculated by liquid volume

Protein carbonyl content (μmol/mL)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)]\div V_{Sample}$**$=0.454\times\Delta A$**

Where: $\Delta A=A_{Test}-A_{Control}$; V_{Total}: Total reaction volume, 0.3 mL; ε: Carbonyl molar extinction coefficient, 22 L/mmol/cm; d: 96-well UV 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. Micro quartz cuvette calculation formula

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

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. For your safety and health, please wear a lab coat and disposable gloves.