

Protein Carbonyl Colorimetric Assay Kit

Catalogue No.: K120

Size: 48T(48S)/ 96T(96S)

Kit component:

Item	Component	Size (48T)	Size (96T)	Storage
Reagent 1	Extraction Buffer	50 mL	100 mL	2-8°C
Reagent 2	Antioxidant	powder*1vial	powder*1vial	2-8°C, shading light
Reagent 3	Chromogen	6 mL	12 mL	2-8°C, shading light
Reagent 4	HCl	6 mL	12 mL	2-8°C
Reagent 5	TCA	15 mL	30 mL	2-8°C
Reagent 6	Guanidine Hydrochloride	30 mL	60 mL	2-8°C

Storage:

The kit should be stored at 2-8°C shading light for 12 months.

Principle of the Assay:

The carbonyl content of the oxidized protein increases, and the carbonyl group can react with 2,4-dinitrophenylhydrazine to form a reddish-brown precipitate, as shown in the figure. After the precipitate is dissolved, the absorbance value at 370 nm can be read on the spectrophotometer to calculate the carbonyl content of the protein.



When testing the sample, it is necessary to determine the protein concentration, and it is recommended to use BCA Protein Assay Kit (K001).

The kit can be used to detect protein carbonyl in serum, plasma, animal and plant tissues, cells, bacteria samples.

Additional Materials Required:

Microplate reader(360-385 nm), Benchtop centrifuge, Constant temperature water bath/incubator
96-well plate, Precision Micropipettor and clean disposable tips, Clean EP tubes

Deionized water, ethyl alcohol, ethyl acetate

Assay Procedure (For reference):

1. Reagent preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

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°C, protected from light.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

HCl: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

TCA: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Guanidine Hydrochloride: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

2. Sample preparation

a. Preparation of sample

Serum and plasma: detect directly.

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.

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. Add samples and test

(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 (μ L)	Test Tube (μ L)
Sample	60	60
Chromogen	0	120
HCl	120	0
Mix well, 37°C , react in darkness for 1 h		
TCA	150	150

Keep it still for 5 min. 4°C , 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°C , 12,000 g centrifuge for 10 min, discard supernatant and keep the precipitation, repeat 3 times

Guanidine Hydrochloride	300	300
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Mix by vortex, incubate at 37°C for 15 min till the precipitate was completely dissolved. 4°C , 12,000 g, centrifuge for 15 min. Take 200 μ L supernatant and add into 96-well plate or microglass cuvette, record the absorbance at 370 nm. Calculate $\Delta A = A_{\text{Test}} - A_{\text{Control}}$.

Calculation:

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

$$\text{Protein carbonyl content } (\mu\text{mol/mg prot}) = [\Delta A \times V_{\text{Total}} \div (\epsilon \times d)] \div (V_{\text{Sample}} \times C_{\text{pr}}) = \mathbf{0.454 \times \Delta A \div C_{\text{pr}}}$$

2. Calculated by sample weight

$$\text{Protein carbonyl content } (\mu\text{mol/g fresh weight}) = [\Delta A \times V_{\text{Total}} \div (\epsilon \times d)] \div (W \times V_{\text{Sample}} \div V_{\text{Total Sample}}) = \mathbf{0.454 \times \Delta A \div W}$$

3. Calculated by cells or bacteria number

$$\text{Protein carbonyl content } (\mu\text{mol}/10^4) = [\Delta A \times V_{\text{Total}} \div (\epsilon \times d)] \div (500 \times V_{\text{Sample}} \div V_{\text{Total Sample}}) = \mathbf{0.454 \times \Delta A \div 500}$$

4. Calculated by liquid volume

$$\text{Protein carbonyl content } (\mu\text{mol/mL}) = [\Delta A \times V_{\text{Total}} \div (\epsilon \times d)] \div V_{\text{Sample}} = \mathbf{0.454 \times \Delta A}$$

Where: $\Delta A = A_{\text{Test}} - A_{\text{Control}}$; 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; $V_{\text{Total Sample}}$: Extraction Buffer volume added, 1 mL; C_{pr} : 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 BCA Assay (Cat #:K001).

Notes:

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing a lab coat and latex gloves during the experiment.

4. The detection range of the kit is not equivalent to the concentration range of the substance to be measured in the sample. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. FineTest will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.