（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Elabscience® Protein Carbonyl Colorimetric Assay Kit

(Tissue And Serum Samples)

Catalog No：E-BC-K117-S

Specification：50Assays(25 samples)/100Assays(50 samples)

Measuring instrument：Spectrophotometer（360-385 nm）

Detection range: 0.02-10 nmol/mgprot

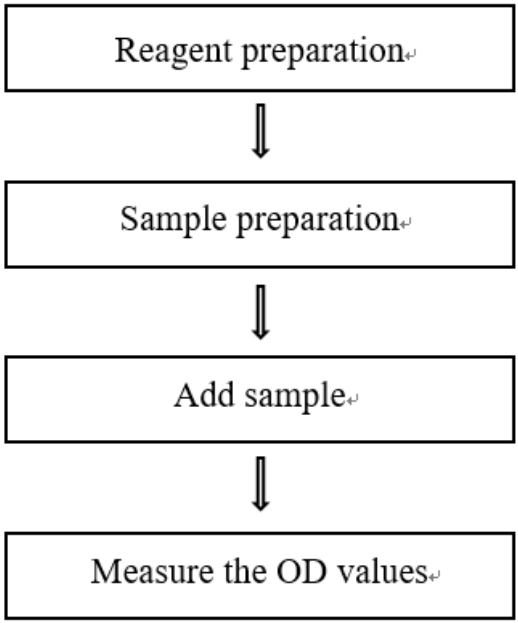
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Phone: 240-252-7368(USA)
Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary

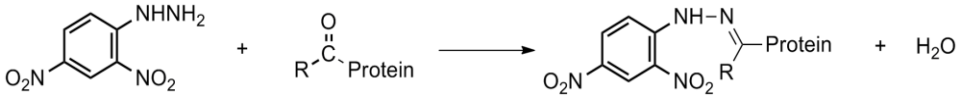


Intended use

This kit can be used for detection of protein carbonyl content in serum (plasma), tissue, hydrothorax, cell culture supernatant samples.

Detection principle

The content of protein carbonyl increased after oxidation, and the carbonyl group reacted with 2, 4-dinitrophenylhydrazine to form a reddish brown precipitate. The absorbance can be measured at 370 nm after the precipitation is dissolved. The carbonyl content can be calculated indirectly.



Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Homogenate Medium	50 mL ×1 vial	50 mL ×2 vials	2-8 ℃, 12 months
Reagent 2	Sulfates	Powder ×1 vial	Powder ×2 vials	2-8 ℃, 12 months, shading light
Reagent 3	DNPH Solution	10 mL ×1 vial	20 mL ×1 vial	2-8 ℃, 12 months, shading light
Reagent 4	Acid Reagent	10 mL ×1 vial	20 mL ×1 vial	2-8 ℃, 12 months
Reagent 5	Protein Precipitator	30 mL ×1 vial	60 mL ×1 vial	2-8 ℃, 12 months
Reagent 6	Denaturant	37.5 mL ×2 vials	50 mL ×3 vials	2-8 ℃, 12 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (360-385 nm, optimum wavelength: 370 nm), Vortex mixer, Micropipettor, Water bath, Incubator, Centrifuge

Reagents:

Double distilled water, deionized water, anhydrous ethanol, ethyl acetate

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of sulfates application solution:
Dissolve one vial of sulfates with 3 mL of double distilled water, Mix well to dissolve. Store at 2-8℃ for 3 days protected from light.

③ The preparation of anhydrous ethanol-ethyl acetate mixture application solution:
For tube well, prepare 1000 μL of anhydrous ethanol-ethyl acetate mixture application solution (mix well 500 μL of anhydrous ethanol and 500 μL of ethyl acetate). The ethanol-ethyl acetate mixture application solution should be prepared on spot.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Hydrothorax sample: Collect fresh hydrothorax sample into the tube which has anticoagulant, centrifuge at 10000 g for 10 min at 4℃ and take supernatant to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

Cell culture supernatant: Collect the fresh cell culture supernatant, centrifuge at 10000×g for 10 min at 4℃ and take supernatant to preserve it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL homogenate medium with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	8-10
Mouse serum	8-10
10% Rat liver tissue homogenization	2-3
10% Mouse heart tissue homogenization	1
10% Mouse brain tissue homogenization	1

Note: The diluent is homogenate medium. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① When washing the precipitate with anhydrous ethanol-ethyl acetate mixture application solution, the vortex must be sufficient. The mixing time should not be less than 1 min and the precipitate must be washed to white. If the precipitate still appear yellow, increase the washing times properly of anhydrous ethanol-ethyl acetate mixture application solution to ensure the washing process is sufficient. Otherwise the result will be higher.

② The speed of centrifuge should not be reduced, otherwise the result will be higher.

③ It is recommended that the round bottom test tube instead of the tip bottom tube should be used to ensure fully washing of the precipitate.

④ The protein content of the samples should be ranged from 1-10 mg/mL.

⑤ Don’t discard the supernatant, it needs to detect the protein content after detect the sample.

⑥ The protein content of the samples can’t be determined using the Bradford method.

Operating steps

1. Sample pretreatment

① Serum (plasma), hydrothorax, cell supernatant: Detect the sample directly.

② Tissue sample: Take 0.45 mL the supernatant and add 0.05 mL of sulfates application solution. Stand for 10min at room temperature, centrifuge at 11580 g for 10 min at 4 ℃ and take the supernatant for detection.

2. The measurement of samples

① Sample tube: Add 0.1 mL of sample, 0.4 mL of DNPH solution into 2 mL EP tubes.

Control tube: Add 0.1 mL of sample, 0.4 mL of acid reagent into 2 mL EP tubes.

② Mix fully by swirling for 1 min and incubate for 30 min at 37 ℃ with shading light.

③ Add 0.5 mL of protein precipitator, mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4 ℃, discard the supernatant and keep the precipitate.

④ Add 1 mL of anhydrous ethanol-ethyl acetate mixture application solution, mix fully by swirling for 1 min, centrifuge at 13780 g for 10 min at 4 ℃, discard the supernatant and keep the precipitate.

⑤ Repeat the step 4 for 3 times (If the precipitate still appear yellow, increase the washing times properly of anhydrous ethanol-ethyl acetate mixture application solution to ensure the washing process is sufficient).

⑥ Add 1.25 mL of denaturant, mix fully by swirling and incubate at 37 ℃ water bath for 15 min accurately.

⑦ Mix fully by swirling to dissolve the precipitate fully. Centrifuge at 13780 g for 15 min at 4 ℃.

⑧ Set the spectrophotometer to zero with reagent 6 and measure the OD values of each tube at 370 nm with 0.5 cm optical path quartz cuvette. Meanwhile, determine the protein concentration of supernatant (Don’t use the Bradford

method to detect the protein concentration, E-BC-K318-M and E-BC-K165-S are recommended).

Calculation

The sample:

$$\text{Protein carbonyl content (nmol/mgprot)} = \frac{A_1\text{-}A_2}{\varepsilon \times d} \div (C_{pr} \times \frac{V_1}{V_2}) \times 10^6 \times f$$

[Note]

A_1: the OD value of sample.

A_2: the OD value of control.

ε: the molar extinction coefficient of carbonyl, 22000 L/mol/cm.

d: the optical path of cuvette, 0.5 cm.

V_1: the total volume of reaction system, 1.25 mL.

V_2: the volume of sample added to the reaction system, 0.1 mL.

C_{pr}: the protein concentration of the sample supernatant, mgprot/L

10^6: unit conversion, 1 mol =10^9 nmol

f: dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mgprot)	1.50	3.80	7.90
%CV	4.8	4.3	4.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mgprot)	1.50	3.80	7.90
%CV	8.4	8.5	8.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (nmol/mgprot)	2.4	5.2	8.5
Observed Conc. (nmol/mgprot)	2.4	5.4	8.6
Recovery rate (%)	99	103	101

Sensitivity

The analytical sensitivity of the assay is 0.02 nmol/mgprot. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

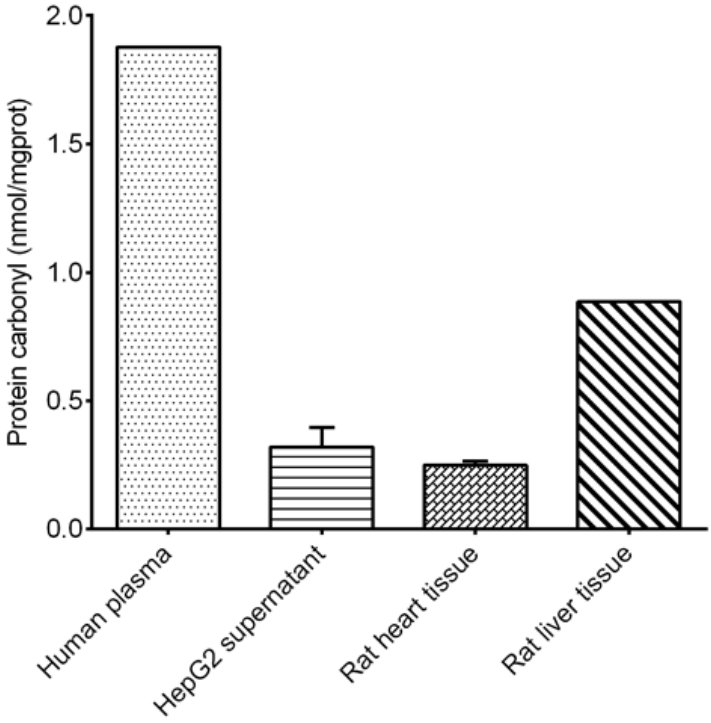
Example analysis:

Take 0.45 mL of rat heart tissue homogenate, add 0.05 mL of sulfates application solution. Stand for 10 min at room temperature, centrifuge at 11580 g for 10 min at 4℃ and take 0.1 mL of the supernatant and carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.027, the average OD value of the control is 0.015, the concentration of protein in sample is 336.10 mgprot/L, and the calculation result is:

$$\text{Protein carbonyl content (nmol/mgprot)} = \frac{0.027 - 0.015}{22000 \times 0.5} \div (336.1 \times \frac{1.25}{0.1}) \times 10^9 = 0.26 \text{ (nmol/mgprot)}$$

Detect human plasma (dilute for 10 times, the concentration of protein in sample is 445.07 mgprot/L), HepG2 cell culture supernatant (the concentration of protein in sample is 272.23 mgprot/L), 10% rat heart tissue homogenate (the concentration of protein in sample is 336.1 mgprot/L) and 10% rat liver tissue homogenate (dilute for 2 times, the concentration of protein in sample is 353.14 mgprot/L) according to the protocol, the result is as follows:


Protein carbonyl (nmol/mgprot)
2.0
1.5
1.0
0.5
0.0
Human plasma
HepG2 supernatant
Rat heart tissue
Rat liver tissue

Statement

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 lab coat and latex gloves.
4. 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. Elabscience 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.