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

Catalog No：E-BC-K171-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（365-375 nm）

Detection range：1.29-45 μg/mL

Elabscience® Total Carbonyl Colorimetric Assay Kit

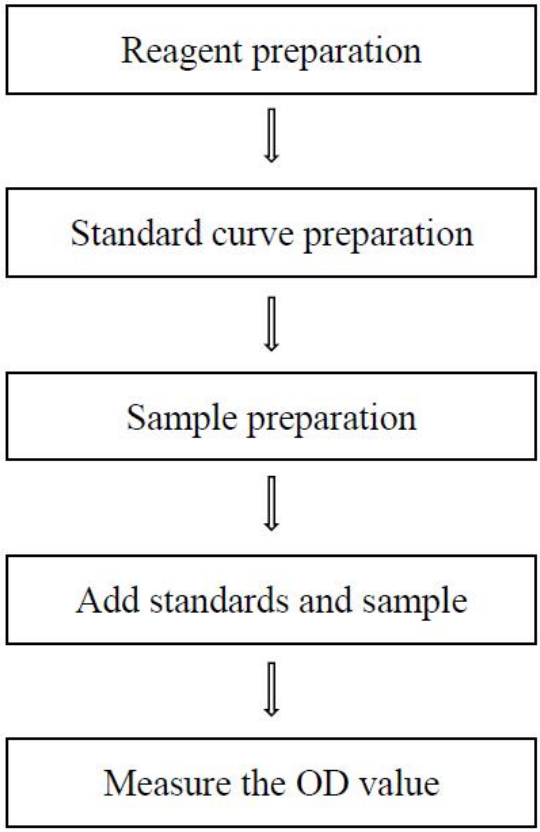
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
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

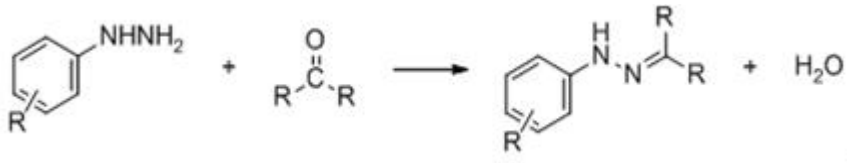


Intended use

This kit can be used for detection of total carbonyl content in serum, plasma and tissue samples.

Detection principle

Carbonyl can react with 2,4-dinitrophenylhydrazine and produce a kind of reddish brown hydrazone compounds, which has a specific absorbance peak at 370 nm. The content of carbonyl can be calculated according to the absorbance value.



Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent Stock Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 2	100 μg/mL Standard	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

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:

Microplate reader (365-375 nm, optimum wavelength: 370 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer.

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic working solution：

For each well, prepare 175 μL of chromogenic working solution (mix well 25 μL of chromogenic agent stock solution and 150 μL of double distilled water). The chromogenic working solution can be stored at 2-8°C for 7 days.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μg/mL standard with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 40, 45 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**5**	**10**	**20**	**25**	**30**	**40**	**45**
100 μg/mL standard (μL)	0	10	20	40	50	60	80	90
Double distilled water (μL)	200	190	180	160	150	140	120	110

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°C for a month.

Tissue samples:

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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

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

② Dilution of sample

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

Sample type	Dilution factor
Porcine serum	1-2
Human serum	1-2
Human plasma	1-2
10% Rat kidney tissue homogenate	3-5
10% Rat liver tissue homogenate	3-5
10% Epipremnum aureum tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The supernatant of sample must be clarified.

② If the samples are frozen used, centrifuge at 10000×g for 10 min and take the supernatant for measurement.

Operating steps

① Standard well: add 24 μL of standard with different concentrations into standard wells.
Sample well: add 24 μL of sample into sample wells.

② Add 175 μL of chromogenic working solution to each well.

③ Mix fully for 5 s with microplate reader and stand for 5 min at room temperature.

④ Measure the OD values of each well at 370 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$\text{Total carbonyl content } (\mu g/mL) = (\Delta A_{370} - b) \div a \times f$$

2. Tissue sample:

$$\text{Total carbonyl content } (\mu g/g) = (\Delta A_{370} - b) \div a \div c \times f$$

[Note]

ΔA_{370}: the absolute OD (OD_{sample}-OD_{blank})

f: dilution factor of the sample before tested.

c: the content of sample = the wet weight (g) ÷ the volume of homogenized medium (mL).

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 (μg/mL)	5.50	19.70	34.50
%CV	2.3	2.0	1.4

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 (μg/mL)	5.50	19.70	34.50
%CV	5.1	4.6	5.3

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 101%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	8.5	22.5	37.4
Observed Conc. (μg/mL)	8.6	23.2	37.0
Recovery rate (%)	101	103	99

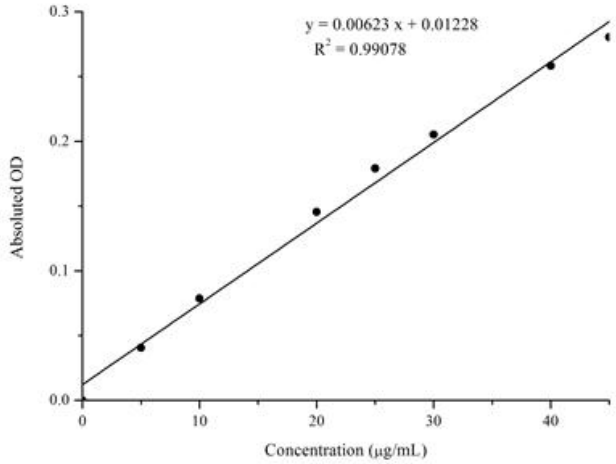
Sensitivity

The analytical sensitivity of the assay is 1.29 μg/mL. 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.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (μg/mL)	0	5	10	20	25	30	40	45
Average OD	0.454	0.495	0.533	0.600	0.634	0.660	0.713	0.735
Absoluted OD	0	0.041	0.079	0.146	0.180	0.206	0.259	0.281



Appendix II Example Analysis

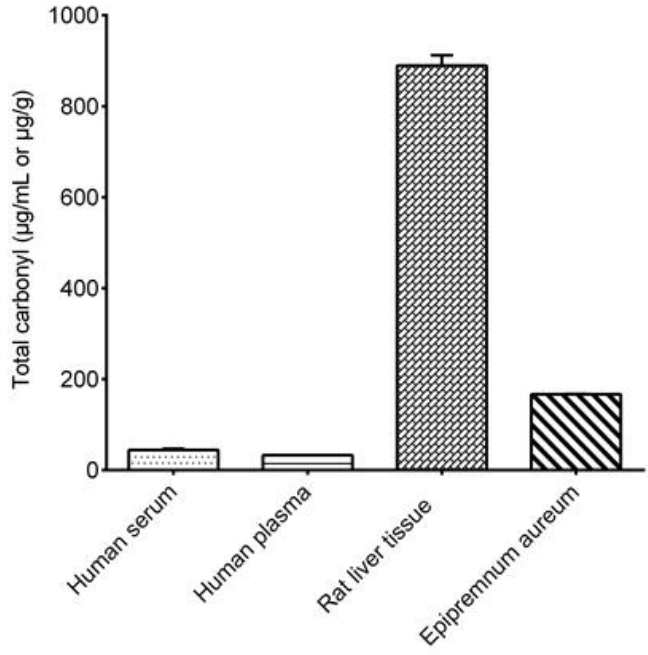
Example analysis：

Take 24 μL of human serum and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.00623 x + 0.01228, the average OD value of the sample is 0.729, the average OD value of the blank is 0.444, and the calculation result is:

$$\text{Total carbonyl content (μg/mL)} = (0.729 - 0.444 - 0.01228) \div 0.00623 = 43.78\ \text{μg/mL}$$

Detect human serum, human plasma, rat kidney tissue homogenate (the content of sample is 0.111 g/mL, dilute for 3 times) and epipremnum aureum tissue homogenate (the content of sample is 0.111 g/mL) according to the protocol, the result is as follows：



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.