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

Catalog No：E-BC-K579-M

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

Measuring instrument：Microplate reader (400-410 nm)

Detection range：0.3-168.4 U/L

Elabscience® Carboxylesterase (CES) Activity 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.

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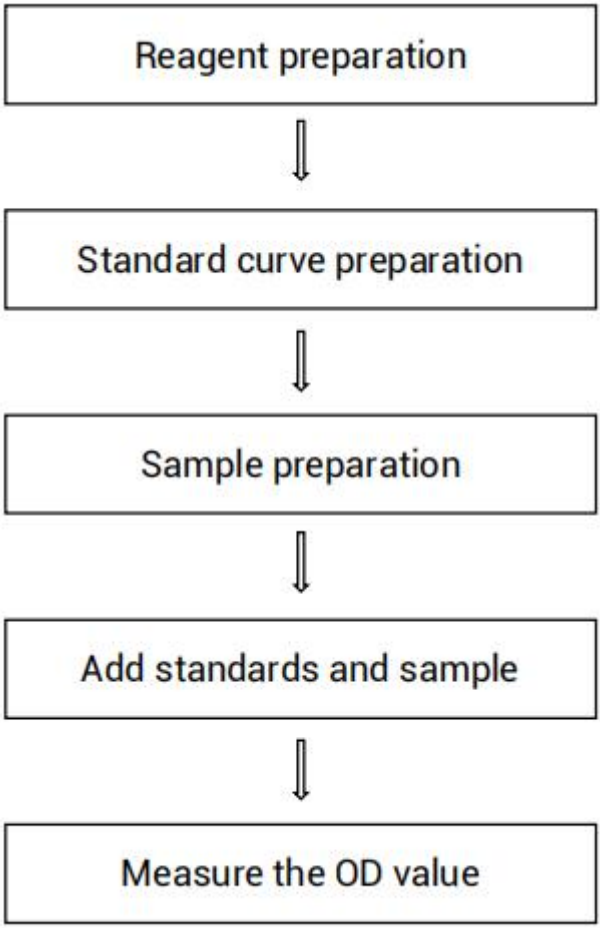
Assay summary



Intended use

This kit can be used to detect the carboxylesterase (CES) activity in animal tissue, cells and serum (plasma) samples.

Detection principle

Carboxylesterase (CES) is a polymeric protein that can catalyze the hydrolysis of carboxylate, thioester, carbamate and amide esters. CES can release effective drugs from some insoluble carboxylate prodrugs through hydrolysis reaction according to its hydrolysis characteristics. In addition, due to its high catalytic efficiency and high specificity, CES can also regulate various metabolic functions (metabolism of ester substances, gene expression, material transport and detoxification, etc.). Therefore, detecting the activity of CES is of great significance for the early diagnosis of cancer and the research of biomedicine.

CES catalyzed substrate reaction to produce chromogenic substance has maximum absorption at wavelength 405 nm, and CES enzyme activity is calculated by measuring OD value at 405 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer	55 mL × 1 vial	55 mL × 2 vials	-20°C, 12months,
Reagent 2	Substrate	0.75 mL ×1 vial	1.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	10 mmol/L Standard Solution	1 mL × 1 vial	1 mL × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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 (400-410 nm, optimum wavelength: 405 nm), Incubator (37°C)

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of 2.5 mmol/L standard solution：

For each well, prepare 1000 μL of 2.5 mmol/L standard solution (mix well 750 μL of double distilled water and 250 μL of 10 mmol/L standard solution). The 2.5 mmol/L standard solution should be prepared on spot protected from light and used up within 1 day.

③ The preparation of working solution：

For each well, prepare 180 μL of working solution (mix well 171 μL of buffer and 9 μL of substrate). The working solution should be prepared on spot protected from light and used up within 1 day.

④ The preparation of standard curve：

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

Dilute 2.5 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.50, 0.75, 1.00, 1.50, 1.75, 2.00, 2.50 mmol/L.

Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.50**	**0.75**	**1.00**	**1.50**	**1.75**	**2.00**	**2.50**
2.5 mmol/L Standard (μL)	0	40	60	80	100	140	160	200
Double distilled water (μL)	200	160	140	120	100	60	40	0

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 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 buffer with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C 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).

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL buffer with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C 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
10% Mouse liver tissue homogenization	100-300
10% Mouse kidney tissue homogenization	100-300
10% Rat liver tissue homogenization	100-300
10% Mouse brain tissue homogenization	10-20
10% Mouse heart tissue homogenization	10-20
Rats serum	10-20
Rats plasma	10-20
Human serum	10-20
Mouse serum	10-20
Mouse plasma	10-20
1×10^6 293T cell	1
1×10^6 Hela cell	1
1×10^6 Jurkat cell	1

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

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

② Add 180 μL of working solution to each well.

③ Mix fully with microplate reader for 5 s and measure the OD value of sample well at 405 nm, as A_1.

④ Incubate at 37°C for 15 min. Measure the OD value of each well as A_2. (The standard curve is fitted to the standard well in A_2 value).

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:

Tissue or cell sample:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol product at 37 °C is defined as 1 unit.

$$\text{CES activity (U/gprot)} = (\Delta A_{405} - b) \div a \div T \times f \div C_{pr} \times 1000$$

Serum and plasma sample

Definition: The amount of enzyme in 1 L plasma or serum per 1 min that produce

1 μmol product at 37 °C is defined as 1 unit.

$$\text{CES activity (U/L)} = (\Delta A_{405} - b) \div a \div T \times f \times 1000$$

[Note]

ΔA_{405}: The OD value of sample well change, $A_2 - A_1$.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

T: Reaction time, 15 min.

1000: 1 mmol/L= 1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rats 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 (U/L)	0.04	0.06	0.08
%CV	5.1	1.8	3.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.04	0.06	0.08
%CV	11.0	2.7	4.0

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.138	0.222	0.295
Observed Conc. (U/L)	0.145	0.222	0.297
Recovery rate (%)	105	100	101

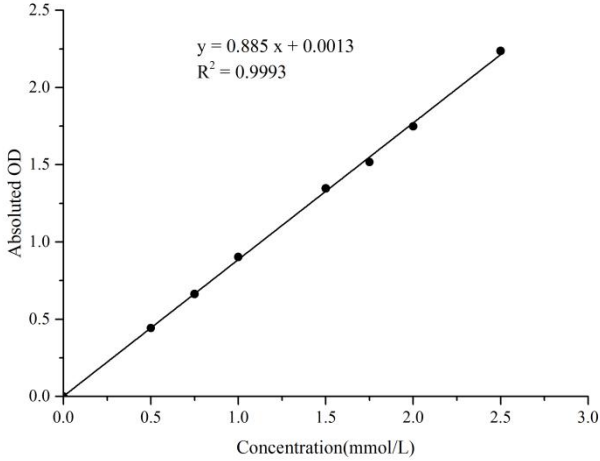
Sensitivity

The analytical sensitivity of the assay is 0.3 U/L. 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 (mmol/L)	0	0.50	0.75	1.00	1.50	1.75	2.00	2.50
OD value	0.097	0.543	0.778	1.006	1.444	1.621	1.878	2.308
	0.099	0.542	0.762	1.002	1.446	1.617	1.848	2.336
Average OD	0.098	0.542	0.762	1.002	1.446	1.617	1.848	2.336
Absoluted OD	0	0.443	0.663	0.903	1.347	1.518	1.749	2.237



Appendix Π Example Analysis

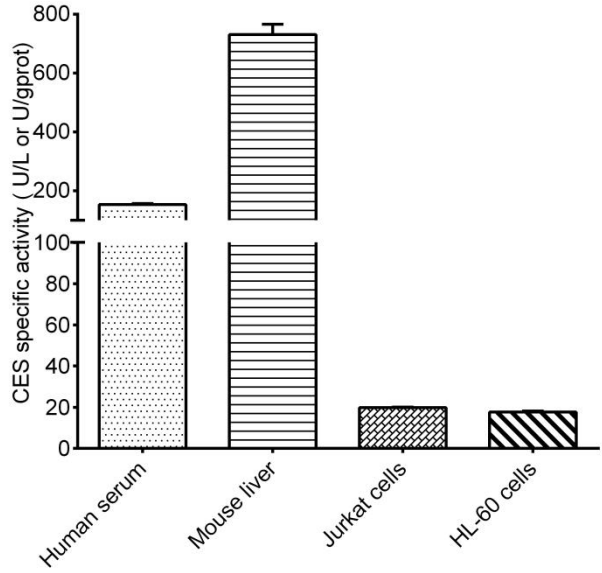
Example analysis：

Take 20 μL of 10% mouse liver tissue homogenization which dilute for 200 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.885 x + 0.0013. The A_1 of sample is 0.209, the A_2 of sample is 0.990, $\Delta A_{405} = A_2 - A_1 = 0.990 - 0.209 = 0.781$, the concentration of protein is 15.067 gprot/L, and the calculation result is:

CES activity(U/gport) = (0.781 - 0.0013) ÷ 0.885 ÷ 15 × 200 ÷ 15.067 × 1000 = 779.6 U/gport

Detect Human serum (dilute for 10 times), 10% mouse liver tissue homogenization (the concentration of protein is 15.067 gprot/L, dilute for 200 times), 1.7×10^6 Jurkat cells (the concentration of protein is 0.555 gprot/L) and 1.87×10^6 HL-60 cells (the concentration of protein is 0.941 gprot/L), 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.