

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

Carboxylesterase (CarE) Assay Kit

Catalog No.: BC00186

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Carboxylesterase (CarE) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell, bacteria
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (450 nm)

Product Introduction

Mammalian carboxylesterase (CarE) is widely distributed in tissues and organs. Most carboxylesterases belong to the serine hydrolase family. CarE catalyzes the hydrolysis of endogenous and exogenous substances containing ester bonds, amide bonds and thioester bonds, but cannot hydrolyze acetylcholine and its analogues. CarE participates in lipid transport and metabolism, and is involved in the detoxification and metabolism of various drugs, environmental toxicants and carcinogens. Organophosphorus pesticides can bind to and inhibit CarE activity.

Principle

CarE catalyzes the hydrolysis of 1-naphthyl acetate to produce naphthol, which develops color with fast blue salt. The absorbance rate is detected at 450 nm to monitor the production rate of naphthol, and thereby calculate CarE activity.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	50 mL×1	Store at 4°C
Reagent 2	Liquid	30 mL×2	Store at 4°C
Reagent 3	Powder	2 vials	Store at 4°C. Before use, take one vial of Reagent 3 and fully dissolve it with 1.2 mL absolute ethanol.
Reagent 4	Powder	2 vials	Store at 4°C

Working solution preparation: Before use, take one bottle of Reagent 2. Add an appropriate amount of Reagent 2 to fully dissolve one vial of Reagent 4. Subsequently, add the dissolved Reagent 4 and one vial of Reagent 3 into the bottle of Reagent 2, mix thoroughly, and filter the mixture. Store at 4°C away from light; the working solution is stable for one week.

Storage

The kit has a validity period of 3 months.

Preparation

Visible spectrophotometer, low-temperature centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, absolute ethanol and distilled water.

- **Sample handling**

1. Preparation of bacterial and cell samples: Collect bacteria or cells into a centrifuge tube and discard the supernatant after centrifugation. Add 400 μ L of Reagent 1 per 2 million bacteria or cells, and lyse the samples by ultrasonication (power: 20%, ultrasonication for 3 s, interval for 10 s, repeated 30 times). Centrifuge at 12,000 g for 30 minutes at 4°C, and collect the supernatant for subsequent detection.
2. Tissue sample treatment: Prepare the mixture at a tissue mass (g) to extract volume (mL) ratio of 1:5-10. It is recommended to weigh approximately 0.1 g of tissue and homogenize it with 1 mL of Reagent 1 under ice-bath conditions. Centrifuge at 12,000 g for 30 minutes at 4°C, and collect the supernatant for subsequent detection.
3. Liquid samples: Use the original sample directly for testing.

Operation process

Before formal determination, be sure to select 2-3 samples with expected large differences to conduct a preliminary experiment.

1. Preheat the spectrophotometer for at least 30 min, adjust the wavelength to 450 nm, and zero the instrument with distilled water.
2. Preheat the working solution at 37°C for no less than 30 minutes.

3. Operation table:

	Blank tube	Assay tube
Distilled water (μL)	5	
Sample (μL)		5
Preheated working solution (μL)	1000	1000
Add the above reagents into a 1 mL cuvette, mix thoroughly, record the absorbance A_1 at 10 s and absorbance A_2 at 3 min 10 s at 450 nm, and calculate $\Delta A = A_2 - A_1$ (the reaction time is 1 minute).		

Calculation

1. CarE activity in tissues:

a. Calculated based on the protein concentration of the sample.

Definition: One unit (U) is defined as the amount of enzyme that causes an increase of 1 in absorbance per milligram of tissue protein per minute at 37°C.

Calculation formula:

$$\text{CarE activity (U/mg)} = \frac{\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}} \times V_{\text{Total}}}{\text{Cpr} \times V_{\text{Sample}}} \div T$$

b. Calculated based on sample fresh weight.

Definition: One unit (U) is defined as an absorbance increase of 1 per gram of tissue per minute at 37°C.

Calculation formula:

$$\text{CarE activity (U/g)} = \frac{\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}} \times V_{\text{Total}}}{W \div V_{\text{Supernatant}} \times V_{\text{Sample}}} \div T$$

2. CarE activity in cells or bacteria

Definition: One unit (U) is defined as an absorbance increase of 1 per 10,000 cells or bacteria per minute at 37°C.

Calculation formula:

$$\text{CarE activity (U/10}^4 \text{ cells)} = \frac{\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}} \times V_{\text{Total}}}{\text{Cell density} \div V_{\text{Supernatant}} \times V_{\text{Sample}}} \div T$$

3. CarE activity in liquid samples

Definition: One unit (U) is defined as an absorbance increase of 1 per mL sample per minute at 37°C.

Calculation formula:

$$\text{CarE activity (U/mL)} = \frac{\Delta A_{\text{Assay}} - \Delta A_{\text{Blank}} \times V_{\text{Total}}}{V_{\text{Sample}}} \div T$$

Annotation:

V_{Total} : Total volume of reaction system, 1.005 mL

V_{Sample} : Volume of sample added, 0.005 mL

$V_{\text{Supernatant}}$: Volume of supernatant added, 1 mL

T: Reaction time, 3 min

Cpr: Sample protein concentration, mg/mL

W: Sample mass, g

Cell density: Total number of cells or bacteria, 10^4 cells/mL

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.