Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Carboxylesterase (CarE) Activity Assay Kit

Cat #: KTB2301 Size: 48 T/96 T

	Micro Carboxylesterase (CarE) Activity Assay Kit		
REF	**Cat #:** KTB2301	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Mammalian CarE also known as aliphatic esterase (aliesterase) is widely distributed in tissue and organism, belonging to the serine hydrolase family. CarE catalyze hydrolysis of endogenous and exogenous substances containing ester bonds, amide bonds, and thioester bonds, but can't catalyze hydrolysis of acetylcholine and its analogues. CarE take part in lipid transport and metabolism, and related with detoxification and metabolism of many drugs, environmental poisons and carcinogens. Organophosphorus pesticides can bind to CarE and inhibit CarE activity. CarE can catalyze acetic acid-1-naphthalene ester to produce naphthalene ester, solid blue color development. Determination of 450 nm light absorption increase rate could calculate CarE activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	15 mL	15 mL×2	4℃
Reagent Ⅱ	1	1×2	4℃, protected from light
Reagent Ⅲ	1	1×2	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Water bath, ice maker, freezing centrifuge
- Deionized water, absolute ethanol
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

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

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅱ: Ready to use as supplied; Take a Reagent Ⅱ and add 0.6 mL absolute ethanol to it to fully dissolve it for use. Reagent Ⅱ is freshly prepared.

Reagent Ⅲ: Ready to use as supplied; Take a Reagent Ⅲ and add a small amount of Reagent Ⅰ to it to fully dissolve it for use. Store at -20℃, protected from light.

Working Reagent: Ready to use as supplied; Add one each of dissolved Reagent Ⅱ and Reagent Ⅲ to a bottle of Reagent Ⅰ, fully dissolve and filter into an empty bottle. (48T with 1 empty bottle, 96T with 2 empty bottles) Store at 4℃ for a week, protected from light. Reagent Ⅲ is freshly prepared.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 12,000 g for 30 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plasma, Serum or other Liquid samples: Test directly.If the solution has turbidity, centrifuge and take the supernatant for measurement.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm. Visible spectrophotometer was returned to zero with deionized water.
2. According to the experimental dosage, part of Working Reagent was preheated at 37℃ for 30 min.
3. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Test Well (μL)
Deionized water	5	0
Sample supernatant	0	5
Working Reagent	200	200

4. Mix thoroughly, detect the absorbance at 450 nm as A_1 after 10 s and A_2 after 190 s. Finally calculate $\Delta A=A_2-A_1$.

Note: The reaction temperature should be kept at 37℃. The general enzyme labeling instrument has the function of temperature control, which can be directly set to 37℃. The experiment can be started when the temperature rises to 37℃.In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is too small, we can appropriately increase the sample size or prolong the reaction time to detect after 5 min or 10 min. If ΔA is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

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.

Calculation of the CarE activity

(1) Calculated by protein concentration

Active unit definition: An increase of 1 per minute of catalytic absorbance per mg tissue protein in 37℃ reaction system is defined as one unit of enzyme activity.

CarE(U/mg prot)==ΔA×V_{Total}÷(Cpr×V_{Sample})÷T**=13.67×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: An increase of 1 per minute of catalytic absorbance per gram tissue in 37℃ reaction system is defined as one unit of enzyme activity.

CarE(U/g fresh weight)==ΔA×V_{Total}÷(Cpr×V_{Sample})÷T**=13.67×ΔA÷Cpr**

(3) Calculated by volume of liquid samples

Active unit definition: An increase of 1 per minute of catalytic absorbance per milliliter of liquid samples in 37℃ reaction system is defined as one unit of enzyme activity.

CarE (U/mL)=ΔA×V_{Total}÷(V_{Sample}×W÷$V_{Total\ sample}$)÷T**=13.67×ΔA**

V_{Total}: total reaction volume, 0.205 mL; V_{Sample}: sample volume added, 0.005 mL; Cpr: sample protein concentration, mg/mL; $V_{Total\ Sample}$: the volume of adding Extraction Buffer, 1 mL; W: sample weight, g; T: reaction time, 3 min.

Precautions

1. If the Working Reagent turns brown, it is considered to be deteriorated and is not available.
2. In order to ensure the accuracy of the reaction time, it is recommended to compare the color one by one; if you want to use a 96-well plate to detect multiple samples at the same time, it is recommended to use a row gun, and a maximum of 8 or 12 holes (8 or 12 rows of guns).

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

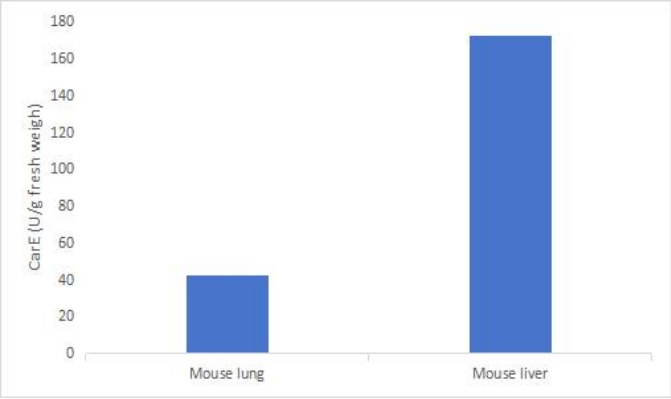

Figure 1. Determination of CarE activity in mouse lung and liver by this kit.

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase(α-GC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.