Carboxylesterase (CarE) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/Microplate reader

Cat No: BC0845

Size: 100T/96S

Components:

Extract solution: 110 mL×1, store at 2-8℃.

Reagent I: Powder×2, store at 2-8℃. Before use, add 1.25 mL anhydrous ethanol, shake to make most of it dissolve, and then add 8.75 mL Reagent III, shake until fully dissolved. The reagent that can not be used up is stored at 2-8℃ for 1 week.

Reagent II: Powder×2, store at -20℃. Before use, 8 mL of Reagent III is added into a bottle of Reagent II, which is fully dissolved by shaking. The reagent is light yellow after dissolving, and it is unstable at room temperature. It is recommended to take the appropriate Reagent II according to the sample size and put it on the ice for use. The remaining reagent should be stored at -20℃ after being sub packed. It is forbidden to freeze and thaw repeatedly.

Reagent III: 40 mL×1, store at 2-8℃.

Description:

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.

Required but not provided:

Spectrophotometer/Microplate Reader, Micro Glass Cuvette/96 Well Flat-Bottom plate, Low Temperature Centrifuge, Water-Bath, Adjustable Pipette, Distilled Water, Anhydrous Ethanol.

Procedure:

I. Sample preparation

1. Bacteria or cells: Collect bacteria or cells to centrifuge tube, discard supernatant. It is suggested that 2 million cells or bacteria should be added to the 400 μL of Extract solution. Ultrasonic crush cells (powder 20%, ultrasonic 3s, interval 10s, repeat 30 times); Centrifuge at 15000 rpm for 10 minutes at 4℃, take the supernatant and placed on ice for testing.
2. Tissue: Tissue(g)：Extract solution=1:5-10(It is suggested that 0.1 g of tissue should be added to the 1mL Extract solution). Homogenate on ice. Centrifuge at 15000 rpm for 10 minutes at 4℃, take the

supernatant and placed on ice for testing.

3. Serum: Directly detect.

II. Detection

1. Preheat spectrophotometer/mcroplate rader for 30 minutes, adjust wavelength to 450 nm, te spectrophotometer needs to be zeroed with distilled water

2. Preheat Reagent I at 37℃ for more than 10 minutes, Reagent II should be placed on ice for use during the detection.

3. Perform the following operations in a micro glass cuvette or 96 well flat-bottom plate:

Reagent Nme (μL)	Blank Tbe (B)	Test Tbe (T)
Distilled water	10	-
Supernatant/serum	-	10
Reagent II	120	120
Reagent I	70	70

Add the above reagents into the cuvette/96 well flat-bottom plate according to the sequence, start timing immediately after adding the Reagent I, blow and mix quickly, record the absorbance value of the 10s as $A1_B$ and $A1_T$, quickly put the cuvette together with the reaction solution in a 37℃ water bath for accurate reaction for 5 min, then quickly take out the cuvette and wipe it dry, record the absorbance at 310s as $A2_B$ and $A2_T$. Calculate $\Delta A_B = A2_B - A1_B$, $\Delta A_T = A2_T - A1_T$. (Blank tube only needs to be done once or twice).

(Note: the environment of 37℃ should be maintained during the reaction of this kit. Generally, the microplate reader has the function of temperature control, which can be directly set to 37℃. The test can be started after the temperature rises to 37℃; if the spectrophotometer without temperature control device is used, the constant temperature water bath can be used to assist the reaction. The specific operation is as follows: add the above reagents into the cuvette according to the sequence, and add Reagent I and start timing, quickly blow and mix, record the absorbance value A1 of the 10s, quickly put the cuvette together with the reaction solution in the 37℃ water bath for accurate reaction for 5 min, then quickly take out the cuvette and wipe it dry, record the absorbance A2 at 310s, and calculate ΔA= A2-A1.)

III. Calculation:

According the micro glass cuvette or 96 well flat-bottom plate.

A. Micro glass cuvette

1. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance value of 1 at 37℃ in 1 mL of reaction system every milligram of tissue protein per minute. $CarE(U/mg\ prot) = (\Delta A_T - \Delta A_B) \times V_{RT} \div 1 \div (Cpr \times V_S) \div T \times F = 4 \times (\Delta A_T - \Delta A_B) \div Cpr \times F$

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance value of 1 at 37℃ in 1 mL of reaction system every gram of tissue weight per minute. $CarE(U/g) = (\Delta A_T - \Delta A_B) \times V_{RT} \div 1 \div (W \div V_{RT} \times V_S) \div T \times F = 4 \times (\Delta A_T - \Delta_B) \div W \times F$

3. Bactria or cells amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the

increasing absorbance value of 1 at 37℃ in 1 mL of reaction system every 10 thousand bacteria or cells per minute.CarE(U/10^4cell)= (ΔA_T-ΔA_B) ×V_{RT}÷1÷(200÷V_{CS}×V_S)÷T×F=0.008×((ΔA_T-Δ_B)×F

4. Serum

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance value of 1 at 37℃ in 1 mL of reaction system every milliliter of serum per minute.CarE(U/mL)= (ΔA_T-ΔA_B) ×V_{RT}÷1÷V_S÷T×F=4×(ΔA_T-ΔA_B)×F

V_{ST}: Supernatant total volume, 1 mL;V_S: Sample volume, 0.01 mL; T: Reaction time, 5 minutes; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; 200: Bacteria or cells amount, 2 million; V_{CS}: Add reagent I to cells, 0.4 mL; V_S: Serum volume, 0.01 mL; V_{RT}: Total volume, 0.2 mL.F: Dilution ratio.

B.96 well flat-bottom plate

1. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance value of 0.5 at 37℃ in 1 mL of reaction system every milligram of tissue protein per minute. CarE(U/mg prot)= (ΔA_T-ΔA_B) ×V_{RT}÷0.5÷(Cpr×V_S)÷T×F=8×(ΔA_T-ΔA_B)÷Cpr×F

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance value of 0.5 at 37℃ in 1 mL of reaction system every gram of tissue weight per minute.CarE(U/g)= (ΔA_T-ΔA_B) ×V_{RT} ÷0.5÷(W ÷V_{RT}×V_S)÷T×F=8×(ΔA_T-Δ_B)÷W×F

3. Bactria or cells amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance value of 0.5 at 37℃ in 1 mL of reaction system every 10 thousand bacteria or cells per minute.CarE(U/10^4cell)= (ΔA_T-ΔA_B) ×V_{RT}÷0.5÷(200÷V_{CS}×V_S)÷T × F=0.016×((ΔA_T-Δ_B)×F

4. Serum

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance value of 0.5 at 37℃ in 1 mL of reaction system every milliliter of serum per minute. CarE(U/mL)= (ΔA_T-ΔA_B) ×V_{RT}÷0.5÷V_S÷T×F=8×(ΔA_T-ΔA_B)×F

V_{ST}: Supernatant total volume, 1 mL;V_S: Sample volume, 0.01 mL; T: Reaction time, 5 minutes; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; 200: Bacteria or cells amount, 2 million; V_{CS}: Add reagent I to cells, 0.4 mL; V_S: Serum volume, 0.01 mL; V_{RT}: Total volume, 0.2 mL.F: Dilution ratio.

Note:

1. Reagent II is stored at -20℃ and is unstable at room temperature. Generally, it can be found that the liquid changes from light yellow to light brown after 2-3 hours of storage at normal temperature

(browning of liquid is regarded as deterioration and cannot be used). It is suggested to calculate the dosage of Reagent II according to the sample size before the test. After Reagent II is dissolved, the required amount should be kept on ice for use. The remaining reagent can be stored at -20℃ after being packed separately.

2. Tissue sample usually dilute 10 times before detection.

3. When the absorbance value is greater than 1, it is suggested that the sample be diluted and measured.

Pay attention to multiplying the dilution multiple in the calculation formula.

4. 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 96 well plate to detect multiple samples at the same time, it is recommended to use the blowgun, and the maximum number of holes (8-channel gun or 12 channel gun) is 8 or 12 wells at a time.

Experimental example:

1. Take 0.1 g of mouse liver and add 1 mL of Extract solution for homogenization. Take the supernatant and dilute it 32 times with distilled water. Operate according to the determination steps. Calculate by 96 well plate: $\Delta A_B = A2_B - A1_B = 0.148-0.122 = 0.026$, $\Delta A_T = A2_T - A1_T = 0.457-0.118 = 0.339$

CarE enzyme activity (U/g mass) = $8 \times (\Delta A_T - \Delta A_B) \times W \times F = 8 \times (0.339-0.026) \times 0.1 \times 32 = 801.28$ U/g mass.

2. Take 0.1 g of mouse kidney, add 1 mL of Extract solution for homogenization, take the supernatant and dilute 8 times with distilled water, and then operate according to the determination steps. Use 96 well plate to measure and calculate $\Delta A_B = A2_B - A1_B = 0.148-0.122 = 0.026$, $\Delta A_T = A2_B - A1_B = 0.28-0.124 = 0.156$

CarE enzyme activity (U/g mass) = $8 \times (\Delta A_T - \Delta A_B) \times F = 8 \times (0.156-0.026) \times 0.1 \times 8 = 83.2$ U/g mass.

Related Products:

BC2020/BC2025 Acetylcholinesterase(AchE) Activity Assay Kit

BC2130/BC2135 Acid Phosphatase(ACP) Activity Assay Kit