Carboxylesterase (CarE) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: NA0806

Size: 50T/48S

Components:

Extract solution: Liquid 50 mL×1, store at 4℃.

Reagent I: Powder×1, store at 4℃. Before use, add 3.125 mL anhydrous ethanol, shake to make most of it dissolve, and then add 21.875 ml Reagent III, shake until fully dissolved. The reagent that can not be used up is stored at 4℃.

Reagent II: Powder×1, store at -20℃. Before use, 18 mL of Reagent III is added into 1 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: Liquid 70 mL×1, store at 4℃.

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

Protocol:

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 place it on ice for test.

2. Tissue: Tissue (g): extract solution=1:5-10 (It is suggested that 0.1 g of tissue should be added to the 1 mL of Extract solution). Homogenate on ice. Centrifuge at 15000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for test.

3. Serum: Directly detect.

II. Detection

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 450 nm, set zero 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. Carry out the following operations in 1 mL glass cuvette：

Reagent name (μL)	Blank tube (B)	Test tube (T)
Distilled water	50	
Supernatant/serum		50
Reagent II	600	600
Reagent I	350	350

Add the above reagents into the cuvette 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).

III. Calculation:

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.

$CarE(U/10^4\ cell) = (\Delta A_T - \Delta A_B) \times V_{RT} \div 1 \div (200 \div V_{ST} \times V_{SV}) \div T \times F = 0.008 \times (\Delta A_T - \Delta A_B) \times F$

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.

$CarE(U/mL)= (\Delta A_T-\Delta A_B) \times V_{RT} \div 1 \div V_S \div T \times F=4\times(\Delta A_T-\Delta A_B)\times F$

V_{ST}: Supernatant total volume, 1 mL;
V_S: Sample volume, 0.05 mL;
T: Reaction time, 5 minutes;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g;
200: Bacteria or cells amount, 2 million;
V_{ST}: Extract solution, 0.4 mL;
V_{SV}: Serum volume, 1 mL;
V_{RT}: Total volume, 1 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. Animal tissue sample dilute 10 times before detection.
3. When the absorbance value is greater than 1, it is suggested that the sample be diluted properly and then determined. Pay attention to multiplying the dilution multiple in the calculation formula.
4. It is suggested that the reaction should be kept at 37℃ and colorimetric one by one.