Choline Acetyltransferase (ChAT) Activity Assay Kit (Tissue Samples)

Catalog No: E-BC-K125-S

Method: Colorimetric method

Specification: 100Assays (Can detect 50 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 1.21 U/g wet weight

Detection range: 1.21-40 U/g wet weight

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure choline acetyltransferase (ChAT) activity in animal tissues samples.

▲Background

Choline Acetyltransferase (ChAT, EC 2.3.1.6) is an enzyme for biosynthesis of acetylcholine, which generates acetylcholine by catalyzing acetyl group transfer from acetyl coenzyme A to choline. ChAT is synthesized in the perinuclear body of neurons and transported to neurons through the mechanism of slow axon transport. ChAT is the most specific indicator for monitoring the functional status of cholinergic neurons in central and peripheral nervous systems.

▲Detection principle

Acetyl-CoA can react with choline under the catalysis of choline acetyltransferase (ChAT) to produce coenzyme A (CoA), CoA can combine with the 4, 4-dithiopyridine. The activity of ChAT can be calculated indirectly by measuring the OD value at 324 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	26 mL × 1 vial	2-8℃ , 3 months
Reagent 2	Inhibitor	1.2 mL × 1 vial	-20℃ , 3 months
Reagent 3	Substrate A	Powder × 1 vial	-20℃ , 3 months
Reagent 4	Substrate B	1.2 mL × 2 vials	2-8℃ , 3 months
Reagent 5	Accelerant A	3 mL × 1 vial	2-8℃ , 3 months
Reagent 6	Accelerant B	1.2 mL × 2 vials	-20℃ , 3 months
Reagent 7	Chromogenic Agent	2 mL × 1 vial	2-8℃ , 3 months
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.			

▲Materials prepared by users

Instruments

Spectrophotometer (324 nm), Micropipettor, Centrifuge, Incubator, Water bath, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲Reagent preparation

The preparation of reagent 3 working solution:

Dissolve a vial of powder with 2.4 mL of double distilled water fully. The prepared solution can be stored at -20℃ for 3 months. It is recommended to aliquot reagent 3 working solution into smaller quantities and store at -20℃ .

▲Sample preparation

Preparation of 20% tissue homogenate

Take 0.1-1 g tissue sample, wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Then add 4 times the volume of PBS (0.01 M, pH 7.4) according to the ratio of Weight (g): Volume (mL) =1:4. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.21-40 U/g wet weight).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
20% Mouse brain tissue homogenate	1-2
20% Rat heart tissue homogenate	1
20% Rat liver tissue homogenate	1
20% Mouse kidney tissue homogenate	1
20% Rat lung tissue homogenate	1

Note:The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	324 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.
(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1.Preparation of substrate working solution:

(the prepared substrate working solution must be use out in 3 hours)

	Substrate working solution
Reagent 1 (μL)	210× (2n+2*)
Reagent 2 (μL)	10× (2n+2*)
Reagent 3 working solution (μL)	20× (2n+2*)
Reagent 4 (μL)	20× (2n+2*)
Reagent 5 (μL)	20× (2n+2*)
Reagent 6 (μL)	20× (2n+2*)
Total amount of mixture reagent (μL)	300 × (n+2*)

Note: n refers to the number of sample.

2*: Prepare 2 more tubes of substrate working solution.

Operation procedure

1. Pretreatment

(1) Control tube: add 50 μL of sample into 2 mL EP tube, then incubate in 100℃ water bath for 2 min.
Sample tube: add nothing.

(2) Add 300 μL of substrate working solution (preheated for 5 min) to each tube.

(3) Control tube: add nothing.
Sample tube: add 50 μL of sample.

(4) Mix fully and incubate at 37℃ water bath for 20 min, then incubate in 100℃ water bath for 2 min to stop the reaction.

(5) Add 850 μL of double distilled water to each tube.

(6) Mix fully and centrifuge at 3100 g for 10 min, then take 750 μL of supernatant to the new corresponding 2 mL EP tube for chromogenic reaction.

2. Chromogenic reaction

(1) Add 15 μL of reagent 7 to each tube.

(2) Mix fully and stand at room temperature for 15 min. Set spectrophotometer to zero with ddH_2O and measure the OD value of each tube at 324 nm with cuvettes (1 cm optical path, 2 mm internal diameter).

▲Operation table

1.Pretreatment

	Sample tube	Control tube
Sample (μL)		50
Incubate in 100℃ water bath for 2 min		
Substrate working solution (μL) (preheated for 5 min)	300	300
Sample (μL)	50	
Mix fully and incubate at 37℃ water bath for 20 min, then incubate in 100℃ water bath for 2 min to stop the reaction.		
Double distilled water (μL)	850	850
Mix fully and centrifuge at 3100 g for 10 min, then take 750 μL of supernatant to the new corresponding 2 mL EP tube for chromogenic reaction.		

2.Chromogenic reaction

	Sample tube	Control tube
Supernatant (μL)	750	750
Reagent 7 (μL)	15	15
Mix fully and stand at room temperature for 15 min. Set spectrophotometer to zero with ddH2O and measure the OD value of each tube at 324 nm with cuvettes (1 cm optical path, 2 mm internal diameter).		

▲Calculation

Unit definition:

The ability of transferring 1 nmol acetyl to choline by 1 g of wet weight tissue at 37℃ and pH 7.2 is defined as 1 unit.

Calculation formula

$$\text{ChAT activity U/g wet weight} = \frac{A_2 - A_1}{t \times \varepsilon \times d} \times \frac{V_2}{V_1} \div \frac{m}{V_3}$$

Note:

A_1: the OD value of control

A_2: the OD value of sample

t: the time of enzymatic reaction, 20 min.

ε: 1.98×10^{-5} L/(nmol•cm), the molar extinction coefficient of product at 324 nm.

d: the optical path of cuvette, 1 cm.

V_1: the volume of sample, 50 μL.

V_2: the total volume of reaction, 1200 μL.

V_3: the volume of PBS added in sample preparation step, L.

m: the weight of sample in sample preparation step, g.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	1.21-40 U/g wet weight	Average intra-assay CV (%)	5
Sensitivity	1.21 U/g wet weight	Average inter-assay CV (%)	9.2

▲Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV is 9.2%

$$\overline{x}_T = \frac{\overline{x}_1 + \overline{x}_2 + \overline{x}_3}{3}$$

$$R = \frac{\overline{x}_{max} - \overline{x}_{min}}{\overline{x}_T} \times 100\%$$

$$\overline{R} = \frac{R_1 + R_2 + R_3}{3} \quad (n=3)$$

$\overline{x}_{max}$--- The max values of $\overline{x}_i$

$\overline{x}_{min}$--- The min values of $\overline{x}_i$

$\overline{x}_T$---The average values of $\overline{x}_i$

R_i---The value of each batch number kit

▲Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 5.0%, which was calculated according to the following formula.

$$CV = \frac{S}{\bar{x}} \times 100\%$$

S--- Standard deviation

▲Sensitivity

The volume of sample added to reaction is 50 μL, carry the assay according to the operation table, the minimum detected absorbance is 0.005 (A_2- A_1=0.005), calculate the sensitivity according to the formula in calculation is 1.21 U/g wet weight.

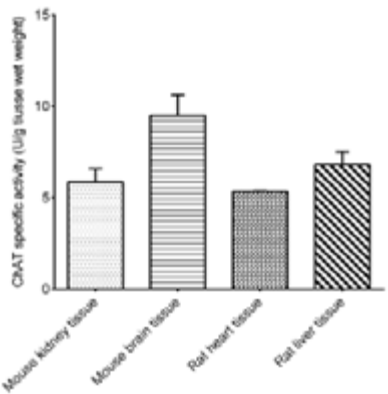
▲Example analysis

Take 50 μL of 20% mouse kidney tissue homogenate sample and carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.067, the average OD value of the control is 0.043, and the calculation result is:

ChAT activity (U/g wet weight)= (0.067-0.043)÷ 20 ÷ 1.98 × 100000 × 1200 ÷ 50 ÷ 0.2 × 0.8 ÷ 1000 = 5.82 U/g wet weight

Detect 20% mouse kidney tissue homogenate, 20% mouse brain tissue homogenate, 20% rat heart tissue homogenate, 20% rat liver tissue homogenate according to the protocol, the result is as follows:



Appendix II References

1. Tuček S. Regulation of Acetylcholine Synthesis in the Brain [J]. Journal of Neurochemistry, 2010, 44(1): 11-24.

2. Oda Y, Nakanishi I. The distribution of cholinergic neurons in the human central nervous system[J]. Histology & Histopathology, 2000, 15(3): 825-834.