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

**Catalog No：E-BC-K125-M**

**Specification：48T(24 samples)/96T(48 samples)**

**Measuring instrument：Microplate reader（324 nm）**

**Detection range: 1.21-40 U/g fresh weight**

# **Elabscience**® Choline Acetyltransferase (ChAT) Activity Assay Kit (Tissue Samples)

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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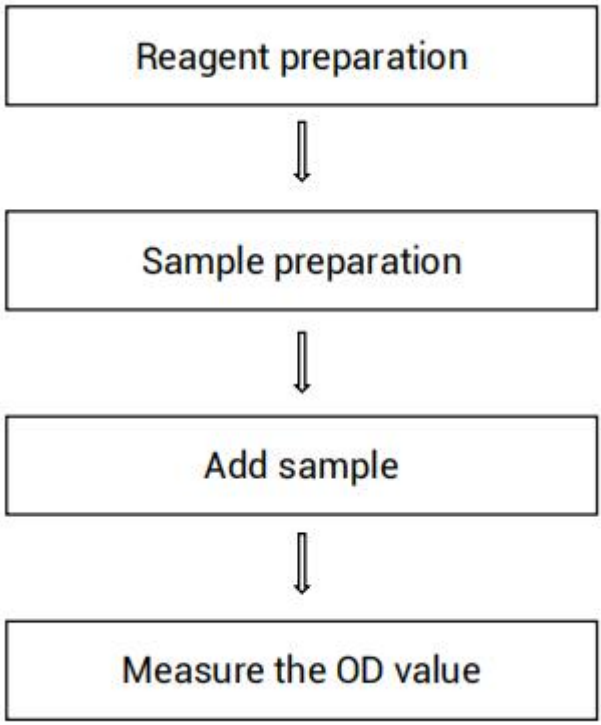
## Assay summary



## Intended use

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

## 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 | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Buffer Solution | 13 mL × 1 vial | 26 mL × 1 vial | 2-8°C, 12 months |
| Reagent 2 | Inhibitor | 1.2 mL × 1 vial | 1.2 mL × 1 vial | -20°C, 12 months |
| Reagent 3 | Substrate A | Powder × 1 vial | Powder × 1 vial | -20°C, 12 months |
| Reagent 4 | Substrate B | 1.2 mL × 1 vial | 1.2 mL × 2 vials | 2-8°C, 12 months |
| Reagent 5 | Accelerant A | 3 mL × 1 vial | 3 mL × 1 vial | 2-8°C, 12 months |
| Reagent 6 | Accelerant B | 1.2 mL × 1 vial | 1.2 mL × 2 vials | -20°C, 12 months |
| Reagent 7 | Chromogenic Agent | 2 mL × 1 vial | 2 mL × 1 vial | 2-8°C, 12 months |
| | UV Microplate | 96 wells | | No requirement |
| | Plate Sealer | 2 pieces | | |
| | Sample Layout Sheet | 1 piece | | |

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

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

**Reagents:**

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

## Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A working solution:
Dissolve one vial of substrate A with 2.4 mL of double distilled water, mix well. Store at -20°C for 3 months. Aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of substrate working solution:
For each tube, prepare 300 μL of substrate working solution (mix well 210 μL of buffer solution, 10 μL of inhibitor, 20 μL of substrate A working solution, 20 μL of substrate B, 20 μL of accelerant A and 20 μL of accelerant B). The substrate working solution should be prepared on spot and used up within 3 hours.

## Sample preparation

**① Sample preparation**

**Tissue sample:**

① Harvest the amount of tissue needed for each assay (initial recommendation
20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 40 mg tissue in 160 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

**② Dilution of sample**

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 |
| 20% Rat heart tissue homogenate | 1 |

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

## Operating steps

**Preteatment**

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

② Add 300 μL of substrate working solution (preheated at 37℃ water both for 5 min) to control tube and sample tube.

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

④ Mix fully and incubate at 37°C water bath for 20 min, then incubate in 100°C 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 3500 g for 10 min, then take 750 μL of supernatant to the new corresponding 2 mL EP tube for chromogenic reaction.

**Chromogenic reaction**

① Add 15 μL of chromogenic agent to each tube.

② Mix fully and stand at room temperature for 15 min. Take 250 μL of supernatant to the corresponding wells of microplate and measure the OD value of each well at 324 nm.

## Calculation

**The sample**:

**Definition:** the ability of transferring 1 nmol acetyl to choline by 1 g of fresh weight tissue at 37°C and pH 7.2 is defined as 1 unit.

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

**[Note]**

$A_1$: the OD value of control

$A_2$: the OD value of sample

t: the time of enzymatic reaction, 20 min.

ε: $1.98\times10^{-5}$ L/(nmol·cm), the molar extinction coefficient of product at 324 nm.

d: the optical path, 0.7 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.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

Three mouse brain tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (U/g fresh weight)** | 2.50 | 18.40 | 34.50 |
| **%CV** | 5.2 | 5.2 | 4.9 |

### Inter-assay Precision

Three mouse brain tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (U/g fresh weight)** | 2.50 | 18.40 | 34.50 |
| **%CV** | 9.5 | 9.4 | 9.3 |

### Sensitivity

The analytical sensitivity of the assay is 1.21 U/g fresh weight. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

## Appendix Π Example Analysis

**Example analysis：**

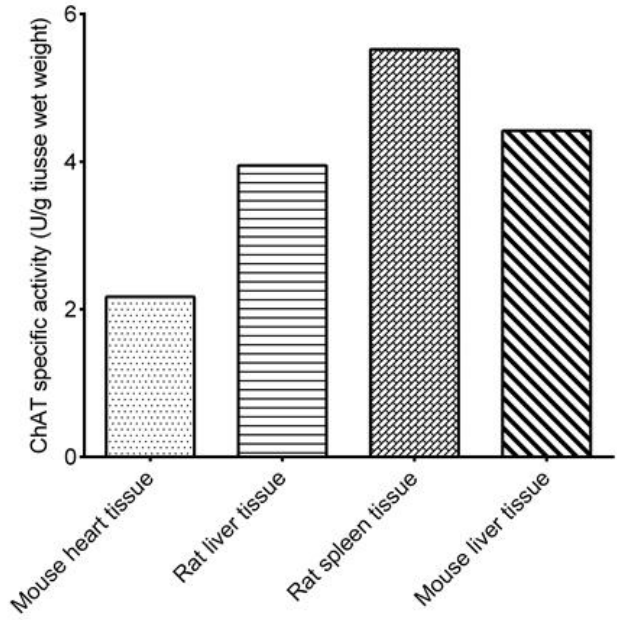
Take 50 μL of 20% mouse heart tissue homogenate sample and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.085, the average OD value of the control is 0.078, and the calculation result is:

$$\text{ChAT activity (U/g fresh weight)} = (0.085-0.078) \div 20 \div 1.98 \div 0.7 \times 100000 \times 1200 \div 50 \div 0.2 \times 0.8 \div 1000$$

$$= 2.42 \text{ U/g fresh weight}$$

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



## Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.