Technical Manual

Choline Acetyltransferase (ChAT) Activity Assay Kit

- Catalogue Code: MAES0101
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

1.21-40 U/g fresh weight

Sensitivity:

1.21 U/g fresh weight

Storage:

2-8°C and -20°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	26 mL × 1 vial	2-8°C, 3 months
Inhibitor	1.2 mL × 1 vial	-20°C, 3 months
Substrate A	Powder × 1 vial	-20°C, 3 months
Substrate B	1.2 mL × 2 vials	2-8°C, 3 months
Accelerant A	3 mL × 1 vial	2-8°C, 3 months
Accelerant B	1.2 mL × 2 vials	-20°C, 3 months
Chromogenic Agent	2 mL × 1 vial	2-8°C, 3 months
UV Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (324 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

After centrifugation, if there is turbidity, centrifuge again.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. The preparation of **substrate A working solution:** Dissolve a vial of substrate A powder with 2.4 mL of double distilled water fully. The prepared solution can be stored at -20°C for 3 months. It is recommended to aliquot substrate A working solution into smaller quantities and store at -20°C.

8. Sample Preparation

Tissue sample:
Take 0.1-1 g tissue sample, wash with PBS (0.01 M, pH 7.4) at 2-8°C. 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.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (1.21-40 U/g fresh 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
20% Rat heart tissue homogenate	1

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

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 324 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

Note: S1-S48, sample wells; S1'- S48', control wells.

10. Operation Steps

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

	Substrate working solution
Buffer Solution (μL)	210× (2n+2*)
Inhibitor (μL)	10× (2n+2*)
Substrate A working solution (μL)	20× (2n+2*)
Substrate B (μL)	20× (2n+2*)
Accelerant A (μL)	20× (2n+2*)
Accelerant B (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.

The measurement of samples

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 for 5 min) to each tube.

3. Control tube: add nothing.

4. Sample tube: add 50 μL of sample.

5. 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.

6. Add 850 μL of double distilled water to each tube.

7. 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.

8. Add 15 μL of chromogenic agent to each tube.

9. 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.

Operation Table

	Sample tube	Control tube
Sample (μL)		50
Incubate in 100°C water bath for 2 min		
Substrate working solution (μL) (preheated for 5 min)	300	300
Sample (μL)	50	
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.		
Double distilled water (μL)	850	850
Mix fully and centrifuge at 3500 g for 10 min, then take supernatant for chromogenic reaction.		
Supernatant (μL)	750	750
Chromogenic agent (μL)	15	15
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.		

11. Calculations

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.

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

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 well, 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

12. Performance Characteristics

Detection Range	1.21-40 U/g fresh weight
Sensitivity	1.21 U/g fresh weight
Average inter-assay CV (%)	9.4
Average intra-assay CV (%)	5.1

Analysis

Take 50 μL of 20% mouse heart tissue homogenate sample, carry the assay according to the operation table.

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:

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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching 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.

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

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