TECHNICAL MANUAL

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

- **SKU CODE:** MAES0101
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Reagent preparation
- Pretreatment
- Chromogenic reaction
- Measure OD value at 324 nm

2. Intended use

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

3. Detection principle

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

4. 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	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. 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)

Note: The reagents must be stored strictly according to the preservation conditions in the table above. The reagents in different kits cannot be mixed with each other. For small volumes of reagents, please centrifuge before use to ensure sufficient reagent recovery.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate A working solution:** Dissolve one vial of substrate A with 2.4 mL of double distilled water and mix well. Store at -20°C for 3 months. Aliquoted storage at -20°C is advised to avoid repeated freeze/thaw cycles.
3. **Preparation of substrate working solution:** For each tube, prepare 300 μL of substrate working solution by mixing 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. Mix well. The substrate working solution should be prepared fresh and used within 3 hours.

7. Sample preparation

1. **Tissue sample preparation:** 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 10,000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.
2. **Sample dilution:** The recommended dilution factors for different samples are as follows (for reference only): 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). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Operating steps

Pretreatment

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°C water bath 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 3,500×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.

9. Calculation

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

ChAT activity (U/g fresh weight) = $(A_2 - A_1) / (t \times \varepsilon \times d) \times (V_2 / V_1) \times (V_3 / m)$

Where:

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, 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

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.

10. Appendix II Example Analysis

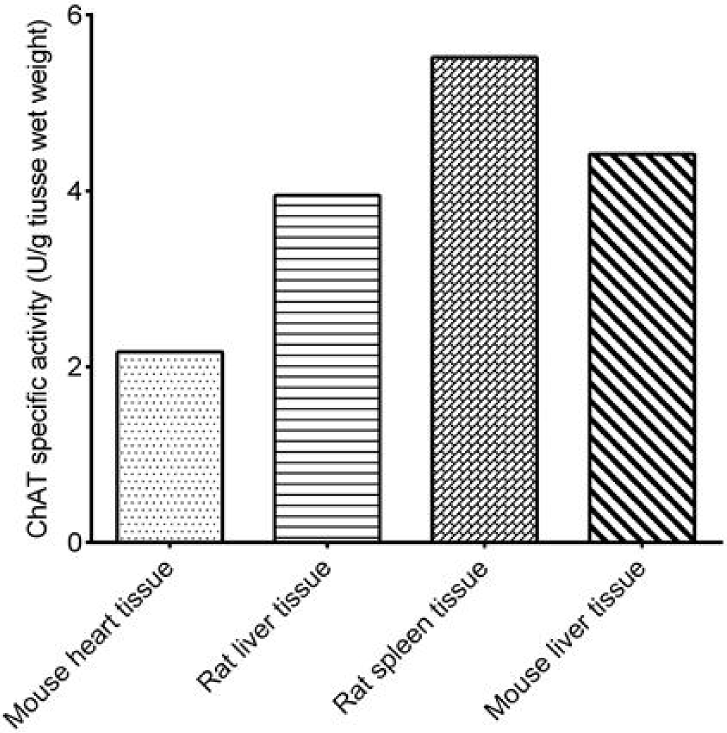
Example analysis:

Take 50 μL of 20% mouse heart tissue homogenate sample and carry out the assay according to the operating 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:

ChAT activity (U/g fresh weight) = (0.085-0.078)÷20÷1.98÷0.7×100000×1200÷50÷0.2×0.8÷1000 = 2.42 U/g fresh weight

Detect 20% mouse heart tissue homogenate, 20% rat liver tissue homogenate, 20% rat spleen tissue homogenate, and 20% mouse liver tissue homogenate according to the protocol. The results are as follows:



11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.