TECHNICAL MANUAL

Acetylcholinesterase (AchE) Activity Assay Kit

- **SKU CODE:** MAES0131
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add sample to microplate
- Add chromogenic working solution
- Add substrate working solution
- Mix and measure absorbance changes at 412 nm

2. Intended use

This kit can be used to measure acetylcholinesterase (AchE) activity in serum, plasma, animal tissue, and cell samples.

3. Detection principle

AchE catalyzes the hydrolysis of acetylcholine to form choline, and choline reacts with dithio p-nitrobenzoic acid (DTNB) to form 5-mercapto-nitrobenzoic acid (TNB). TNB has an absorption peak at 412 nm. The activity of AchE is calculated by measuring the increasing rate of absorbance at 412 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Lysis Buffer	50 mL x 1 vial	50 mL x 2 vials	2-8°C, 12 months
Reagent 2	Buffer Solution	15 mL x 1 vial	30 mL x 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	Powder x 1 vial	Powder x 1 vial	2-8°C, 12 months, protect from light
Reagent 4	Substrate	Powder x 1 vial	Powder x 1 vial	2-8°C, 12 months, protect from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (412 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer.

Reagents:

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

6. Reagent preparation

1. Incubate buffer solution at 37°C for 30 min. Equilibrate other reagents to room temperature before use.
2. **Preparation of chromogenic working solution:** 48T: Dissolve one vial of chromogenic agent with 11 mL of buffer solution, mix well. Store at 2-8°C for 7 days protected from light. 96T: Dissolve one vial of chromogenic agent with 22 mL of buffer solution, mix well. Store at 2-8°C for 7 days protected from light.
3. **Preparation of substrate working solution:** 48T: Dissolve one vial of substrate with 0.7 mL of buffer solution, mix well. Store at 2-8°C for 7 days protected from light. 96T: Dissolve one vial of substrate with 1.3 mL of buffer solution, mix well. Store at 2-8°C for 7 days protected from light.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one month.

Tissue samples:

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 20 mg tissue in 180 µL lysis buffer 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.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 1×10^6 cells in 200 µL lysis buffer with an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

Mouse serum: 8-20

Mouse plasma: 4-10

Human serum: 4-10

Human plasma: 4-10

Rat serum: 4-10

Dog serum: 4-10

Horse serum: 2-8

10% Mouse liver tissue homogenate: 1

10% Mouse kidney tissue homogenate: 1

10% Mouse brain tissue homogenate: 2-8

10% Crucian carp muscle 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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

The samples must not contain chelating agents such as EGTA and EDTA, or reducing substances such as DTT and mercaptoethanol.

9. Operating steps

1. Take 20 µL of sample to the well of microplate.

2. Add 170 µL of chromogenic working solution to each well.

3. Add 10 µL of substrate working solution to each well.

4. Mix fully for 5 s with microplate reader, measure the changes in absorbance at 412 nm within 5 min. The OD value at 30 seconds and 330 seconds were recorded as A_1 and A_2, respectively. ΔA = A_2 - A_1.

10. Calculation

Tissue sample:

① Calculate according to the protein concentration of sample

Definition: The enzymatic amount that catalyzes the production of 1 nmol TNB by 1 mg of protein per minute is defined as 1 unit.

AchE activity (U/mgprot) = $(\Delta A \times V_{total} / (\varepsilon \times d) \times 10^9) \div C_{pr} \times V_{sample} \div T \times f = 245 \times \Delta A \div C_{pr} \times f$

② Calculate according to the weight of sample

Definition: The enzymatic amount that catalyzes the production of 1 nmol TNB by 1 g of sample per minute is defined as 1 unit.

AchE activity (U/g fresh weight) = $(\Delta A \times V_{total} / (\varepsilon \times d) \times 10^9) \div W \times V_{sample} / V_{total}$ sample $\div T \times f = 245 \times \Delta A \div W \times f$

2. Serum (plasma) samples:

Definition: The enzymatic amount that catalyzes the production of 1 nmol TNB by 1 mL of serum (plasma) per minute is defined as 1 unit.

AchE activity (U/mL) = $(\Delta A \times V_{total} / (\varepsilon \times d) \times 10^9) \div V_{sample} \div T \times f = 245 \times \Delta A \times f$

3. Cell sample:

Definition: The enzymatic amount that catalyzes the production of 1 nmol TNB by 1 mg of protein per minute is defined as 1 unit.

AchE activity (U/mgprot) = $(\Delta A \times V_{total} / (\varepsilon \times d) \times 10^9) \div C_{pr} \times V_{sample} \div T \times f = 245 \times \Delta A \div C_{pr} \times f$

[Note]

ε: molar extinction coefficient of TNB, 1.36×10^4 L/mol/cm;

d: optical path of the 96-well microplate, 0.6 cm;

V_{total}: total volume of reaction system, 2×10^{-4} L;

V_{sample}: volume of sample added into the reaction system, 20 μL = 2×10^{-2} mL;

V_{total} sample: volume of the added extraction solution, 1 mL;

10^9: unit conversion, 1 mol = 10^9 nmol;

T: reaction time, 5 min;

W: weight of sample, g;

C_{pr}: concentration of protein in sample, mg/mL;

f: dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	10.20	88.50	264.00
%CV	5.2	4.6	4.3

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	10.20	88.50	264.00
%CV	9.1	9.3	9.5

Recovery

Three samples of high, medium, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 104%.

Recovery

Sample	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	138.5	252	381.3
Observed Conc. (U/mL)	146.8	257.0	396.6

Sample	Sample 1	Sample 2	Sample 3
Recovery rate (%)	106	102	104

Sensitivity

The analytical sensitivity of the assay is 1.225 U/mL. 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.

12. Appendix II Example Analysis

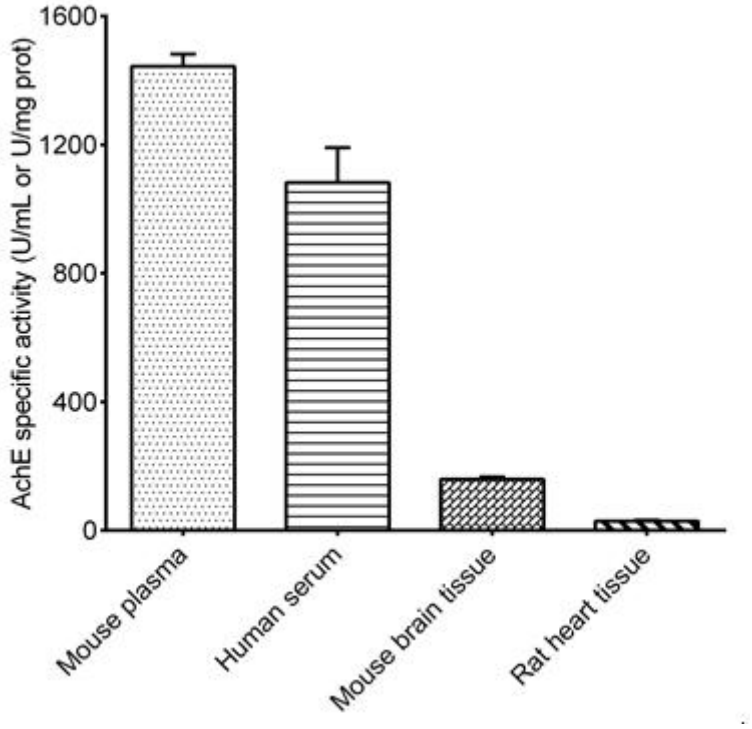
Example analysis:

Dilute rat serum with PBS (0.01 M, pH 7.4) 5-fold, take 20 μL of diluted sample and carry out the assay according to the operating steps. The results are as follows:

The average OD values at 30 s and 330 s are A_1 (0.297) and A_2 (0.405), $\Delta A = A_2 - A_1 = 0.108$, and the calculation result is:

AchE activity (U/mL) = 245 × 0.108 × 5 = 132.3 U/mL

Detect mouse plasma (dilute 5-fold), human serum (dilute 10-fold), 10% mouse brain tissue homogenate (protein concentration is 6.879 mgprot/mL, dilute 3-fold) and 10% rat heart tissue homogenate (protein concentration is 3.410 mgprot/mL) according to the protocol, the results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from using the kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and calibrate the instruments before performing experiments. Please follow the instructions strictly during the experiments.

3. Protection methods must be taken by wearing a lab coat and latex gloves.

4. If the concentration of the substance is not within the detection range, an additional dilution or concentration step should be performed on the sample.

5. It is recommended to perform a pre-test if your sample is not listed in the instruction manual.

6. The 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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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