TECHNICAL MANUAL

Cholinesterase (CHE) Activity Assay Kit

- **SKU CODE:** MAES0210
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add samples to EP tubes
- Incubate at 37°C
- Add chromogenic agents
- Centrifuge and measure OD value

2. Intended use

This kit can be used for detection of cholinesterase (CHE) activity in serum (plasma) and animal tissue samples.

3. Detection principle

Cholinesterase breaks down acetylcholine into choline and acetic acid. Acetylcholine that is not hydrolyzed by cholinesterase reacts with hydroxylamine to form acetamidamine. It reacts in an acidic solution to form a brown-red hydroxamate iron complex. The color depth is directly proportional to the amount of remaining acetylcholine, which can be colorimetrically quantified. Cholinesterase activity is then calculated.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	2-8°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	Diluent A	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8°C, 12 months
Reagent 4	Chromogenic Agent A	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
Reagent 5	Alkaline Reagent	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months
Reagent 6	Acid Reagent	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months
Reagent 7	Protein Precipitator	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 8	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months, shading light
Reagent 9	Diluent B	0.5 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (505-535 nm, optimum wavelength: 520 nm), Centrifuge, Vortex mixer, 37°C water bath

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

Size 1(48 T):

① Equilibrate all reagents to room temperature before use.

② The preparation of 80 μmol/mL substrate stock solution:

Dissolve one vial of substrate powder with 1 mL of diluent A. Mix well to dissolve. Store at 2-8°C for 1 week protected from light.

③ The preparation of substrate application solution:

For each well, prepare 60 μL of substrate application solution (mix well 6 μL of substrate stock solution and 54 μL of buffer solution). The substrate application solution should be prepared on spot and used up in the same day.

④ The preparation of chromogenic agent A stock solution:

Dissolve one vial of chromogenic agent A with 10 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 3 months.

⑤ The preparation of chromogenic agent A application solution:

For each well, prepare 200 μL of chromogenic agent A application solution (mix well 100 μL of chromogenic agent A stock solution and 100 μL of alkaline reagent). The substrate application solution should be prepared on spot and used up in the same day.

⑥ The preparation of diluent B application solution:

Dilute 250 μL of diluent B with 9.75 mL of double distilled water, mix well. Store at 2-8°C for 6 months.

⑦ The preparation of chromogenic agent B application solution:

Dissolve one vial of chromogenic agent B with 10 mL of diluent B application solution. Mix well to dissolve. Store at 2-8°C for 3 months protected from light.

Size 2(96 T):

① Equilibrate all reagents to room temperature before use.

② The preparation of 80 μmol/mL substrate stock solution:

Dissolve one vial of substrate powder with 1 mL of diluent A. Mix well to dissolve. Store at 2-8°C for 1 week protected from light.

③ The preparation of substrate application solution:

For each well, prepare 60 μL of substrate application solution (mix well 6 μL of substrate stock solution and 54 μL of buffer solution). The substrate application solution should be prepared on spot and used up in the same day.

④ The preparation of chromogenic agent A stock solution:

Dissolve one vial of chromogenic agent A with 20 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 3 months.

⑤ The preparation of chromogenic agent A application solution:

For each well, prepare 200 μL of chromogenic agent A application solution (mix well 100 μL of chromogenic agent A stock solution and 100 μL of alkaline reagent). The substrate application solution should be prepared on spot and used up in the same day.

⑥ The preparation of diluent B application solution:

Dilute 500 μL of Diluent B with 19.5 mL of double distilled water, mix well. Store at 2-8°C for 6 months.

⑦ The preparation of chromogenic agent B application solution:

Dissolve one vial of chromogenic agent B with 20 mL of diluent B application solution. Mix well to dissolve. Store at 2-8°C for 3 months protected from light.

7. Sample preparation

① 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 a month.

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 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes 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).

② Dilution of sample

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

Sample type	Dilution factor
Human serum	2-3
Human plasma	2-3
Mouse serum	2-3
Mouse plasma	2-3
10% Rat brain tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat lung tissue homogenate	1

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

8. The key points of the assay

1. The brown-red iron complex after reaction is unstable, and the colorimetry must be completed within 20 minutes.
2. There should be no bubbles in the wells of the microplate when measuring the OD value.

9. Operating steps

1. Blank tube: Take 80 μL of double distilled water to the 2 mL EP tube. Control tube: Take 20 μL of double distilled water and 60 μL of substrate application solution to the 2 mL EP tube. Sample tube: Take 20 μL of sample and 60 μL of substrate application solution to the 2 mL EP tube.
2. Add 150 μL of buffer solution to each tube.

3. Mix fully and incubate at 37°C for 20 min.
4. Successively add 200 µL of chromogenic agent A application solution, 150 µL of acid reagent, 100 µL of protein precipitator, 100 µL of chromogenic agent B application solution to each tube and mix fully.
5. Centrifuge at 2300 g for 10 min.
6. Take 250 µL of supernatant to the corresponding wells of microplate, measure the OD value of each well at 520 nm with microplate reader.

10. Calculation

The sample:

1. Serum (plasma) sample:

Unit definition: The amount of CHE in 1 mL of serum or plasma that react with substrate in 20 minute at 37°C and decompose 1 µmol acetylcholine is defined as 1 unit.

CHE activity (U/mL) = $(\Delta A_1 - \Delta A_2) / \Delta A_1 \times C \times V_1 / V_2 \times f$

2. Tissue sample:

Unit definition: The amount of CHE in 1 mg of tissue protein that react with substrate in 20 minute at 37°C and decompose 1 µmol acetylcholine is defined as 1 unit.

CHE activity (U/mgprot) = $(\Delta A_1 - \Delta A_2) / \Delta A_1 \times C \times V_1 / V_2 \div C_{pr} \times f$

[Note]

ΔA_1: $OD_{Control} - OD_{Blank}$

ΔA_2: $OD_{Sample} - OD_{Blank}$

C: The concentration of substrate application solution, 8 µmol/mL

V_1: The volume of substrate application solution, 0.06 mL

V_2: The volume of sample added to the reaction, 0.02 mL

C_{pr}: Concentration of protein in sample, mgprot/mL

f: Dilution factor of sample before tested

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)	8.50	24.60	58.50
%CV	2.5	2.3	2.4

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)	8.50	24.60	58.50
%CV	5.9	6.8	6.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	16.4	33.6	60.5
Observed Conc. (U/mL)	16.2	34.6	62.9
recovery rate(%)	99	103	104

Sensitivity

The analytical sensitivity of the assay is 2.17 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

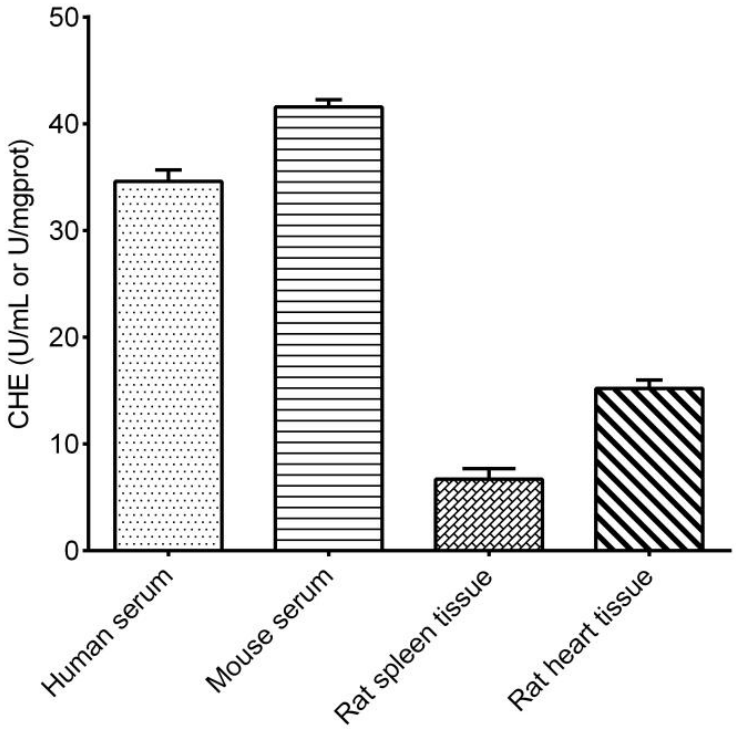
Example analysis:

For human plasma, dilute for 2 times, and carry the assay according to the operation steps. The results are as follows:

the average OD value of the blank is 0.044, the average OD value of the sample is 0.236, the average OD value of the control is 0.426, and the calculation result is:

CHE activity (U/mL) = ((0.426 – 0.044) - (0.236 – 0.044)) ÷ (0.426 – 0.044) × 8 × 0.06 ÷ 0.02 × 2= 23.85 U/mL

Detect human serum (dilute for 2 times), mouse serum (dilute for 2 times), 10% rat spleen tissue homogenate (the concentration of protein is 8.60 mgprot/mL) and 10% rat heart tissue homogenate (the concentration of protein is 4.52 mgprot/mL) according to the protocol, the result is 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 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.

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.