TECHNICAL MANUAL

Choline/Acetylcholine Fluorometric Assay Kit

- **SKU CODE:** MAES0278
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Operating steps
- Measure fluorescence intensity
- Calculation

2. Intended use

This kit can be used to measure choline/acetylcholine content in serum, plasma, and animal tissue samples.

3. Detection principle

Choline and acetylcholine play important roles in many biological processes. Choline is an essential nutrient, often grouped with B complex vitamins, that plays a key role in many biological processes. Choline is a precursor to the synthesis of acetylcholine (AChE), an important neurotransmitter in the peripheral and central nervous systems.

Choline is measured by enzyme coupling reaction to generate fluorescent products in this kit, and the content of fluorescent substances is proportional to the presence of choline. Acetylcholinesterase is added to the reaction to hydrolyze acetylcholine into choline and acetic acid, and the acetylcholine content is further calculated by measuring the total amount of choline and acetylcholine.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Hydrolase	1.3 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 3	Complex Enzyme	0.4 mL × 1 vial	-20°C, 12 months, protect from light

Item	Component	Size (96 T)	Storage
Reagent 4	1 mmol/L Standard Solution	0.1 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 5	Chromogenic Agent	0.1 mL × 1 vial	-20°C, 12 months, protect from light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37°C).

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. **Equilibrate all reagents to room temperature before use.:** The hydrolase and complex enzyme should be aliquoted for storage at -20°C, and repeated freeze/thaw cycles should be avoided.
2. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the test wells. For example, prepare 123 µL of working solution by mixing 3 µL of complex enzyme with 120 µL of buffer solution. Keep on ice and protect from light during use. The working solution should be used within 4 hours.
3. **Preparation of chromogenic working solution:** Before testing, prepare sufficient chromogenic working solution according to the test wells. For example, prepare 255 µL of chromogenic working solution by mixing 5 µL of chromogenic agent with 250 µL of buffer solution. Keep on ice and protect from light during use. The chromogenic working solution should be used within 4 hours.
4. **Preparation of 10 µmol/L standard solution:** Dilute 10 µL of 1 mmol/L standard solution with 990 µL of buffer solution and mix thoroughly. Store at 2-8°C for 2 days protected from light.

5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 10 µmol/L standard with buffer solution to serial concentrations. The recommended dilution gradient is: 0, 2, 3, 4, 6, 8, 9, 10 µmol/L.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one 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 10,000×g for 10 min to remove insoluble material. Collect supernatant and keep 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):

10% Mouse heart tissue homogenate: 10-20

10% Rat liver tissue homogenate: 10-20

10% Mouse lung tissue homogenate: 10-20

10% Mouse kidney tissue homogenate: 15-25

10% Mouse brain tissue homogenate: 15-25

10% Mouse liver tissue homogenate: 10-20

Human serum: 3-5

Rat serum: 3-7

Rat plasma: 1

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

8. The key points of the assay

The hydrolase and complex enzyme should avoid repeated freeze/thaw cycles.

9. Operating steps

The content of choline:

① Standard well: Add 20 μL of different concentration solutions into standard wells.

Sample well (choline): Add 20 μL of samples into sample wells.

Control well: Add 20 μL of samples into control wells.

② Add 120 μL of working solution into standard wells and sample wells (choline). Add 140 μL of buffer solution into control wells.

③ Add 20 μL of buffer solution into standard wells and sample wells (choline).

④ Add 20 μL of chromogenic working solution into each well.

⑤ Mix thoroughly with microplate reader for 3 s and incubate at room temperature for 5 min protected from light. Measure the fluorescence intensity of each well at excitation wavelength of 535 nm and emission wavelength of 587 nm.

2. The content of choline and acetylcholine:

① Standard well: Add 20 μL of different concentration solutions into standard wells.

Sample well (choline): Add 20 μL of samples into sample wells.

Sample well (choline and acetylcholine): Add 20 μL of samples into sample wells.

Control well: Add 20 μL of samples into control wells.

② Add 120 μL of working solution into standard wells, sample wells (choline) and sample wells (choline and acetylcholine). Add 140 μL of buffer solution into control wells.

③ Add 20 μL of buffer solution into standard wells and sample wells (choline). Add 20 μL of hydrolase into sample wells (choline and acetylcholine).

④ Add 20 μL of chromogenic agent working solution into each well.

⑤ Mix thoroughly with microplate reader for 3 s and incubate at room temperature for 5 min protected from light. Measure the fluorescence intensity of each well at excitation wavelength of 535 nm and emission wavelength of 587 nm.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #1) from all standard readings. This is the absolute fluorescence value.

3. Plot the standard curve using absolute fluorescence value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Tissue sample:

Choline content (μmol/gprot) = (ΔF_1 - b) ÷ a ÷ C_{pr} × f

Acetylcholine content (μmol/gprot) = (ΔF_2 - b) ÷ a ÷ C_{pr} × f

2. Serum and plasma sample:

Choline content (μmol/L) = (ΔF_1 - b) ÷ a × f

Acetylcholine content (μmol/L) = (ΔF_2 - b) ÷ a × f

[Note]

ΔF_1: Absolute F value of choline (F_{Sample} of choline – $F_{Control}$).

ΔF_2: Absolute F value of acetylcholine (F_{Sample} of choline and acetylcholine – F_{Sample} for choline).

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.00	3.00	7.00
%CV	5.0	3.2	3.8

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.00	3.00	7.00
%CV	8.2	10.0	8.8

Recovery

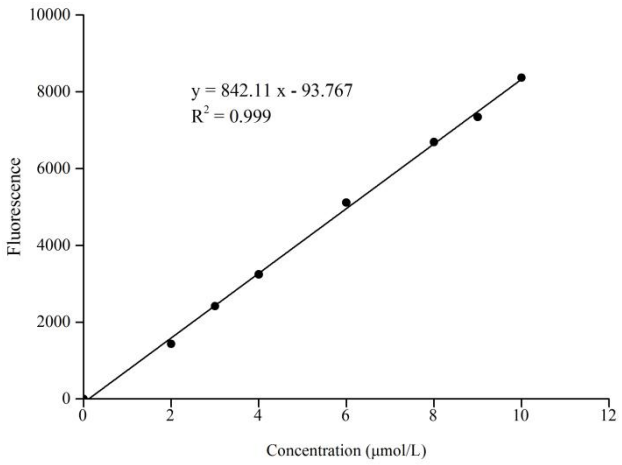
Standard	Expected Conc. (µmol/L)	Observed Conc. (µmol/L)	Recovery rate (%)
Standard 1	2.5	2.5	101
Standard 2	5	5.0	99
Standard 3	8.5	8.5	100

Sensitivity

The analytical sensitivity of the assay is 0.06 µmol/L. 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.

Standard curve

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



12. Appendix II Example Analysis

Example analysis:

For 10% mouse liver tissue homogenate, diluted 20 times, take 20 µL of sample and carry out the assay according to the operation table. The results are as follows:

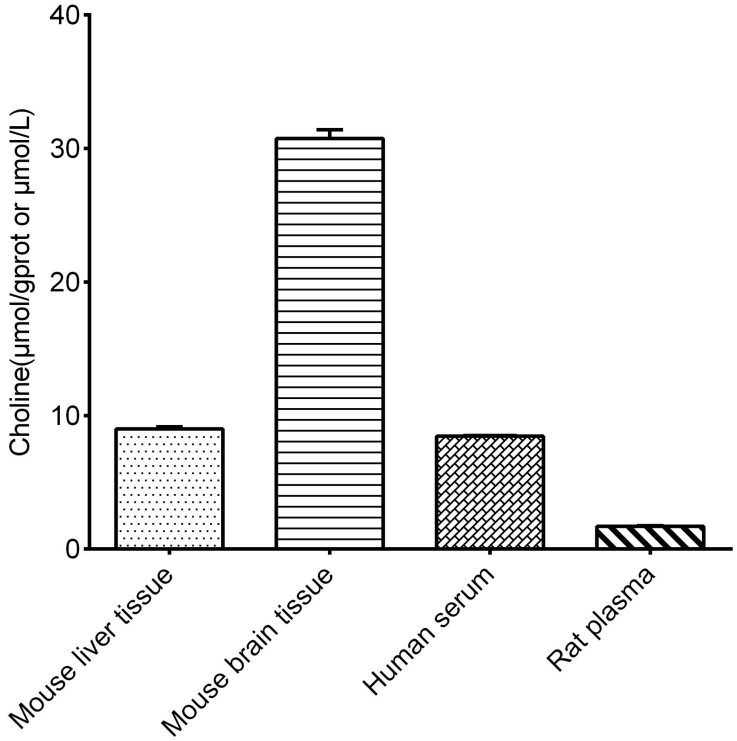
Standard curve: y = 842.11x – 93.767, the average fluorescence value of the control is 358, the average fluorescence value of the sample (choline) is 2684, the average fluorescence value of the sample (choline and acetylcholine) is 3282.

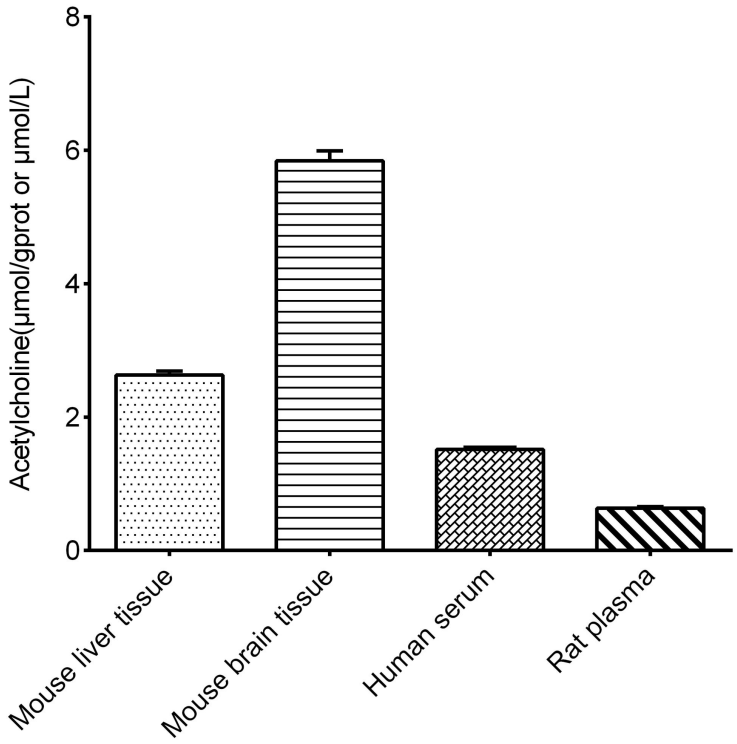
ΔF_1 = 2684 - 358 = 2326, ΔF_2 = 3282 - 2684 = 598, the concentration of protein in sample is 6.33 gprot/L and the calculation result is:

Choline content (μmol/gprot) = (2326 + 93.767) ÷ 842.11 ÷ 6.33 × 20 = 9.07 (μmol/gprot)

Acetylcholine content (μmol/gprot) = (598 + 93.767) ÷ 842.11 ÷ 6.33 × 20 = 2.60 (μmol/gprot)

Detect 10% mouse liver tissue homogenate (protein concentration 6.33 gprot/L, diluted 20 times), 10% mouse brain tissue homogenate (protein concentration 5.59 gprot/L, diluted 20 times), human serum and rat plasma 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 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.