（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F055

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.06-10 μmol/L

Elabscience® Choline/Acetylcholine Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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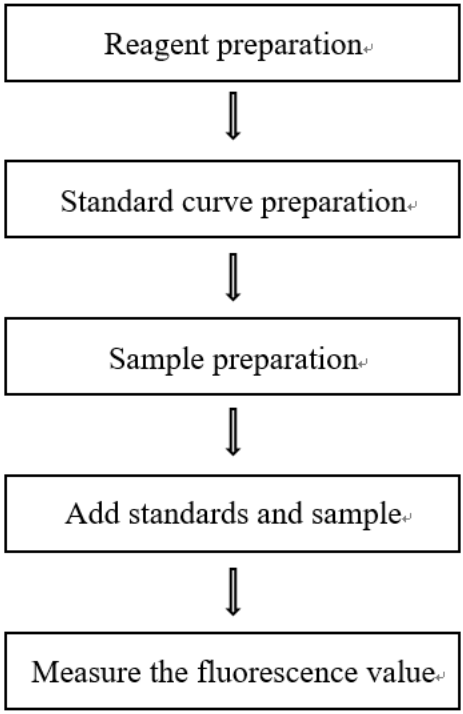
Assay summary



Intended use

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

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 was added to the reaction to hydrolyze acetylcholine into choline and acetic acid, and the acetylcholine content was further calculated by measuring the total amount of choline and acetylcholine.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL ×1 vial	-20℃, 12 months
Reagent 2	Hydrolase	1.3 mL ×2 vials	-20℃, 12 months shading light
Reagent 3	Complex Enzyme	0.4 mL ×1 vial	-20℃, 12 months shading light
Reagent 4	1 mmol/L Standard Solution	0.1 mL ×1 vial	-20℃, 12 months shading light
Reagent 5	Chromogenic Agent	0.1 mL ×1 vial	-20℃, 12 months shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use. The hydrolase and the complex enzyme should be aliquoted storage at -20 ℃, and avoid repeated freeze/thaw cycles is advised.

② The preparation of working solution:

Before testing, please prepare sufficient working solution according to the test

wells. For example, prepare 123 μL of working solution (mix well 3 μL of complex enzyme and 120 μL of buffer solution). Keep it on ice and protected from light during use. The working solution should be used up within 4 hours.

③ The preparation of chromogenic working solution:

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 255 μL of chromogenic working solution (mix well 5 μL of chromogenic agent and 250 μL of buffer solution). Keep it on ice and protected from light during use. The chromogenic working solution should be used up within 4 hours.

④ The preparation of 10 μmol/L standard solution:

Dilute 10 μL of 1 mmol/L standard solution with 990 μL of buffer solution and mix fully. Store at 2-8 ℃ for 2 days protected from light.

⑤ The 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 a serial concentration. The recommended dilution gradient is as follows: 0, 2, 3, 4, 6, 8, 9, 10 μmol/L.

Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**2**	**3**	**4**	**6**	**8**	**9**	**10**
10 μmo/L standard (μL)	0	40	60	80	120	160	180	200
Buffer solution (μL)	200	160	140	120	80	40	20	0

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℃ 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℃.

④ Centrifuge at 10000×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 (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
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 the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

The hydrolase and the complex enzyme should be avoid repeated freeze/thaw cycles is advised.

Operating steps

1. The content of choline:

① Standard well: add 20 μL of different concentrations solution into standard well.
Sample well (choline): add 20 μL of samples into sample well.
Control well: add 20 μL of samples into control well.

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

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

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

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

2. The content of choline and acetylcholine:

① Standard well: Add 20 μL of different concentrations solution into standard well.
Sample well (choline): Add 20 μL of samples into sample well.
Sample well (choline and acetylcholine): Add 20 μL of samples into sample well.
Control well: Add 20 μL of samples into control well.

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

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

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

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

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue sample:

$$\text{Choline content}(\mu\text{mol/gprot}) = (\Delta F_1 - b) \div a \div C_{pr} \times f$$

$$\text{Acetylcholine content}(\mu\text{mol/gprot}) = (\Delta F_2 - b) \div a \div C_{pr} \times f$$

2. Serum and plasma sample:

$$\text{Choline content}(\mu\text{mol/L}) = (\Delta F_1 - b) \div a \times f$$

$$\text{Acetylcholine content}(\mu\text{mol/L}) = (\Delta F_2 - b) \div a \times f$$

[Note]

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

ΔF_2: Absolute F value of acetylcholine ($F_{\text{Sample of choline and acetylcholine}} - F_{\text{Sample for choline}}$).

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

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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 (μmol/L)	1.00	3.00	7.00
%CV	5.0	3.2	3.8

Inter-assay Precision

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

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

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 100%.

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

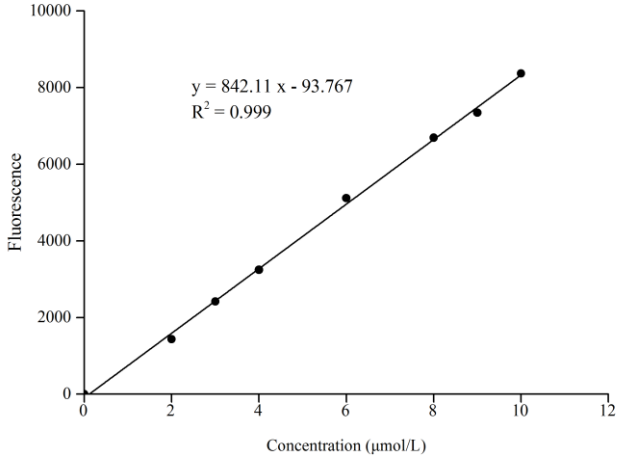
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	2	3	4	6	8	9	10
Fluorescence value	1021	2487	3379	4326	6118	7603	8179	9317
	1083	2485	3565	4265	6220	7886	8611	9524
Average fluorescence value	1052	2486	3472	4296	6169	7745	8395	9421
Absoluted fluorescence value	0	1434	2420	3244	5117	6693	7343	8369



Appendix II Example Analysis

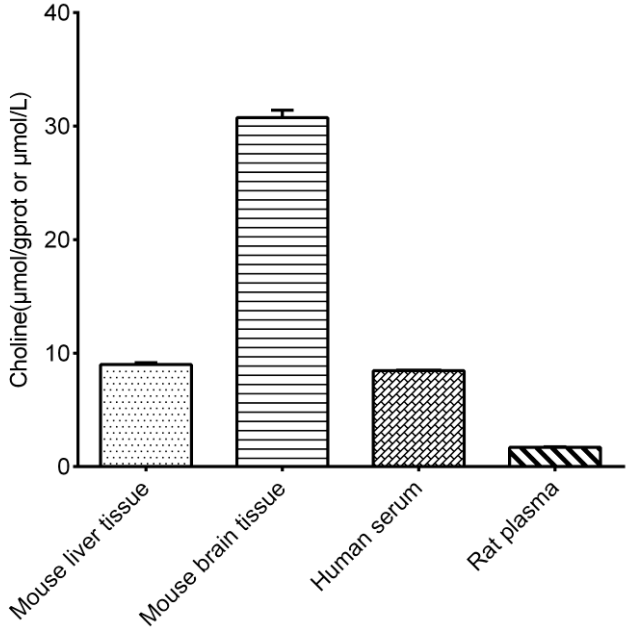
Example analysis:

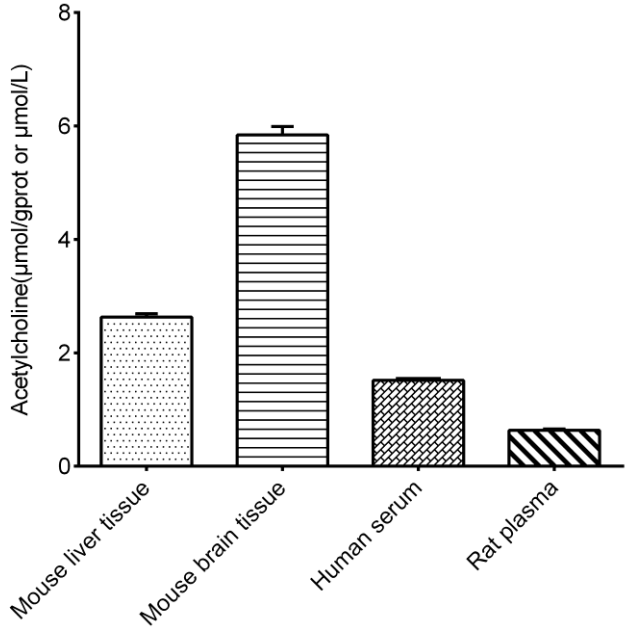
For 10% mouse liver tissue homogenate, dilute for 20 times, take 20 uL of sample and carry the assay according to the operation table. The results are as follows: standard curve: y = 842.11 x – 93.767, the average fluorescence value of the control is 358, the average fluorescence value of the sample (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 (the concentration of protein is 6.33 gprot/L, dilute for 20 times), 10% Mouse brain tissue homogenate (the concentration of protein is 5.59 gprot/L, dilute for 20 times), human serum and rat plasma according to the protocol, the result is as follows:


Choline(μmol/gprot or μmol/L)
40
30
20
10
0
Mouse liver tissue
Mouse brain tissue
Human serum
Rat plasma


Acetylcholine(μmol/gprot or μmol/L)
8
6
4
2
0
Mouse liver tissue
Mouse brain tissue
Human serum
Rat plasma

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.