Technical Manual

Acetylcholinesterase (AchE) Activity Assay Kit

- Catalogue Code: MAES0131
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

1.225-490 U/mL

Sensitivity:

1.225 U/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background:

Acetylcholinesterase (AchE) (EC 3.1.1.7) is a serine hydrolase with monomer, dimer and tetramer structures, also known as G1, G2 and G4, containing one, two or four catalytic subunits, mainly exist in neuromuscular junctions and cholinergic synapses in the brain. The main biological role of AchE is the rapid hydrolysis of the neurotransmitter acetylcholine into acetic acid and choline to terminate the impulsive transmission of cholinergic synapses.

3. Intended Use:

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

4. Detection Principle:

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

5. Kit Components & Storage:

Item	Specification	Storage
Lysis Buffer	50 mL × 2 vials	2-8°C, 6 months
Buffer Solution	30 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Substrate	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (412 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

The samples could not contain chelating agents such as EGTA and EDTA, or reductive substances such as DTT and mercapto ethanol.

7. Reagent Preparation:

1. Incubate buffer solution at 37°C for 30 min.

2. Preparation of **chromogenic agent working solution:** Dissolve a vial of chromogenic agent powder with 22 mL of buffer solution fully before use. The prepared solution can be used for 7 days; avoid direct sunlight.

3. Preparation of **substrate working solution:** Dissolve a vial of substrate powder with 1.3 mL of buffer solution fully before use. The prepared solution can be used for 7 days; avoid direct sunlight.

8. Sample Preparation:

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb water and weigh. Homogenize at the ratio of the volume of lysis buffer (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (1.225-490 U/mL).

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

Sample Type:	Dilution Factor:
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);

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 412 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

Note: S1-S96, sample wells.

10. Operation Steps:

The measurement of samples

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

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

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

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

Operation Table

	Sample well
Sample (μL)	20
Chromogenic agent working solution (μL)	170
Substrate working solution (μL)	10
Mix fully for 5s with microplate reader, measure the changes in absorbance at 412 nm within 5 min. The OD value of 30s and 330s were recorded as A_1 and A_2, respectively. $\triangle A=A_2-A_1$.	

11. Calculations:

1. Serum (plasma) and other liquid sample:

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.

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

2. Tissue sample:

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.

$$\text{AchE activity (}U/mg\ prot\text{)} = (\triangle A \times \frac{V\ total}{\varepsilon \times d} \times 10^9) \div (Cpr \times V\ sample) \div T \times f$$
$$= 245 \times \triangle A \div Cpr \times f$$

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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.

$$\text{AchE activity } (U/g \text{ fresh weight}) = (\triangle A \times \frac{V \text{ total}}{\varepsilon \times d} \times 10^9) \div \frac{W \times V \text{ sample}}{V \text{ total sample}} \div T \times f$$

$$= 245 \times \triangle A \div W \times f$$

ε: molar extinction coefficient of TNB, 13.6×104 L /mol/cm
d: optical path of the 96 wells 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

12. Performance Characteristics

Detection Range	1.225-490 U/mL
Sensitivity	1.225 U/mL
Average recovery rate (%)	104
Average inter-assay CV (%)	9.3
Average intra-assay CV (%)	4.7

Analysis

Dilute rat serum with PBS (0.01 M, pH 7.4) for 5 times, take 20 μL of diluted sample, carry the assay according to the operation table.

The results are as follows:

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

$$\textbf{AchE activity (U/mL)} = 245 \times 0.108 \times 5$$

$$= 132.3 \text{ U/mL}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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Notes:

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