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CheKine™ Micro Acetylcholinesterase (AchE) Activity Assay Kit

Cat #: KTB1710 Size: 96 T

	Micro Acetylcholinesterase (AchE) Activity Assay Kit		
REF	**Cat #:** KTB1710	LOT	**Lot #:** Refer to product label
	Applicable samples: Fresh Serum, Fresh Plasma, Animal Tissues, Nerve Cells		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Acetylcholinesterase (AchE) belongs to serine hydrolase, which is widely present in various animal tissues and serum. AchE catalyzes the hydrolysis of acetylcholine (Ach) and plays an important role in the regulation of nerve conduction. CheKine™ Micro Acetylcholinesterase (AchE) Activity Assay Kit provides a convenient tool for detection of AchE Activity. The principle is that AchE catalyzes the hydrolysis of Ach to produce choline, and choline reacts with dithiopara-nitrobenzoic acid (DTNB) to produce 5-mercapto- Nitrobenzoic acid (TNB), TNB has a maximum absorption peak detected at 412 nm. The enzyme activity of AchE was calculated by detecting the rate of increase in absorption at 412 nm.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer	100 mL	4℃
Assay Buffer	16 mL	4℃
Chromogen	1.3 mL	4℃, protected from light
Substrate	1.3 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Substrate: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Working Reagent: Prepare 180 μL Work Reagent for one well, add 160 μL Assay Buffer, 10 μL Chromogen, 10 μL Substrate. Prepare Work Reagent before use and depend on your need. Working Reagent is freshly prepared.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Nerve Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Serum, Plasma, or other Liquid samples: Tested directly.

Note: For animal tissues with high fat content, remove the upper layer of fat after centrifugation, and then take the supernatant. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm, visible spectrophotometer was returned to zero with deionized water.

2. Preheat the incubator to 37℃. Working Reagent is placed in incubator to preheat for 30 min.

3. Add 20 μL of sample, 180 μL of Working Reagent to the 96-well plate or microglass cuvette, then tap the plate and mix well quickly. Measure absorbance value within 3 min at 412 nm. The 20 s absorbance value is recorded as A_1, then incubate at 37℃ for 3 min and the 200 s absorbance value is recorded as A_2, calculate $\Delta A=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.002, increase the sample quantity appropriately. If ΔA is greater than 0.8, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

1. Calculation the activity of AchE in animal tissues

(1) Calculated by protein concentration

Unit definition: 1 nmol TNB produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

AchE (U/mg prot)=$(\Delta A\div\varepsilon\div d\times V_{Reaction\ Total}\times10^9)\div(Cpr\times V_{Sample})\div T\times n$**=490×ΔA÷Cpr×n**

(2) Calculated by fresh weight of samples

Unit definition: 1 nmol TNB produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

AchE (U/g)=$(\Delta A\div\varepsilon\div d\times V_{Reaction\ Total}\times10^9)\div(W\times V_{Sample}\div V_{Sample\ Total})\div T\times n$**=490×ΔA÷W×n**

2. Calculated the activity of AchE by cells number

Unit definition: 1 nmol TNB produced per min in 10^4 cells reaction system is defined as a unit of enzyme activity.

AchE (U/10^4 cells)=$(\Delta A\div\varepsilon\div d\times V_{Reaction\ Total}\times10^9)\div(\text{Total number of cells}\times V_{Sample}\div V_{Sample\ Total})\div T\times n=490\times\Delta A\div500$**=0.98×ΔA×n**

3. Calculate the activity of AchE in liquid sample

Unit definition: 1 nmol TNB produced per min in 1mL liquid sample reaction system is defined as a unit of enzyme activity.

AchE(U/mL)=(ΔA÷ε÷d×$V_{Reaction\ Total}$×10^9)÷V_{Sample}÷T×n**=490×ΔA×n**

Where: ε: TNB molar extinction coefficient, 13.6×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; $V_{Reaction\ Total}$: total reaction volume, 200 μL=2×10^{-4} L; 10^9: 1 mol=1×10^9 nmol; Cpr: sample protein concentration, mg/mL; V_{sample}: sample volume added, 0.02 mL; T: reaction time, 3 min; n: dilution factor; W: sample weight, g; $V_{Sample\ Total}$: Extraction Buffer volume added, 1 mL; 500: Total number of cells, 5×10^6.

B. Microglass cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Typical Data

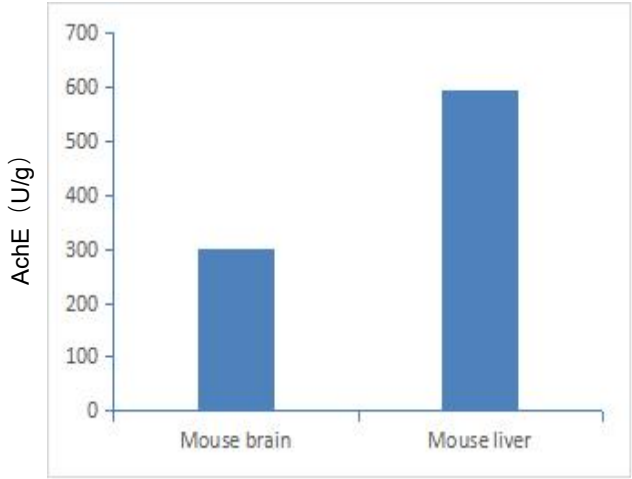

Figure 1. AchE activity in mouse brain, mouse liver respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1700	CheKine™ Micro Tissue and Blood Alkaline Phosphatase (AKP/ALP) Assay Kit
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.