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CheKine™ Micro Acetylcholine (Ach) Content Assay Kit

Cat #: KTB1711 Size: 48 T/24 S 96 T/48 S

	Micro Acetylcholine (Ach) Content Assay Kit		
REF	**Cat #:** KTB1711	LOT	**Lot #:** Refer to product label
	Detection range: 20-3,750 μg/mL		**Sensitivity:** 20 μg/mL
	Applicable sample: Serum and Whole blood samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Acetylcholine (Ach) is a widely existing neurotransmitter in living organisms and plays a crucial role in the nervous system. The CheKine™ Micro Acetylcholine (Ach) Content Assay Kit provides a simple, convenient, and rapid method for detecting Ach levels, suitable for serum and whole blood samples.The principle of the assay is as follows: Ach reacts with the substrate to generate acetylnhydroxamic acid, which forms a brown-colored complex under acidic conditions. The intensity of the color is proportional to the concentration of Ach.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	7 mL	14 mL	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅳ	3 mL	6 mL	4℃
Reagent Ⅴ	3 mL	6 mL	4℃
Reagent Ⅵ	3 mL	6 mL	4℃, protected from light
Reagent Ⅶ	1.5 mL	1.5 mL	4℃
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm

- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, ice maker, vortex mixer
- Deionized water

Reagent Preparation

Working Reagent Ⅰ: Prepared before use. For the 48 T kit, add 1.75 mL deionized water to thoroughly dissolve the powder; for the 96 T kit, add 3.5 mL deionized water. Unused reagent can be aliquoted and stored at -20℃ in the dark for up to 2 weeks. Avoid repeated freeze-thaw cycles.

Reagent Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use. Store protected from light at 4℃.

Working Reagent Ⅲ: Prepared before use. For the 48 T kit, add 3 mL deionized water to thoroughly dissolve the powder; for the 96 T kit, add 6 mL deionized water. Unused reagent can be stored at 4℃ protected from light for up to 1 month.

Working Reagent Ⅳ: Prepared before use. Mix Working Reagent Ⅲ and Reagent Ⅳ at a ratio of 1:1 (v/v). Prepare only the required amount for each experiment and use immediately.

Reagent Ⅴ: Ready to use as supplied; Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅵ: Ready to use as supplied; Equilibrate to room temperature before use. Store protected from light at 4℃.

Reagent Ⅶ: Ready to use as supplied; Equilibrate to room temperature before use. Store at 4℃.

Standard: Prepared before use. Dissolve the powder completely in 1 mL of Reagent Ⅶ to obtain a 5 mg/mL acetylcholine stock solution. The stock solution can be aliquoted and stored at -20℃ in the dark for up to 1 month. Avoid repeated freeze-thaw cycles.

500 μg/mL Standard: Dilute 20 μL of the 5 mg/mL acetylcholine stock solution with 180 μL deionized water. Mix thoroughly. This dilution is stable for one day and should be used within the same day.

Note: Reagent Ⅰ, Reagent Ⅱ, Reagent Ⅲ and Standard are toxic to some extent. It is recommended that all procedures involving these reagents be carried out in a fume hood.

Sample Preparation

Note: Fresh samples are recommended. If not tested immediately, samples can be stored at -80℃ for up to one month. The thawing temperature and time should be controlled during sample preparation. For thawing at room temperature, the process should be completed within 4 h.

1. Whole Blood Samples: Take 200 μL of fresh heparinized whole blood and mix with 350 μL deionized water to induce hemolysis. Add 50 μL of Working Reagent Ⅰ, followed by slow addition of 200 μL Reagent Ⅱ. Mix thoroughly. Centrifuge at 3,500 g for 10 min at 4℃. Collect the supernatant and place it on ice for immediate assay.
2. Serum Samples: Take 200 μL of serum and add 50 μL of Working Reagent Ⅰ, followed by slow addition of 200 μL of Reagent Ⅱ. Mix thoroughly. Centrifuge at 3,500 g for 10 min at 4℃. Collect the supernatant and place it on ice for immediate assay.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are performed in a 1.5 mL EP tube):

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
500 μg/mL Standard	0	8	0	0
Deionized Water	60	52	0	0
Reagent Ⅱ	20	20	0	0
Sample supernatant	0	0	80	80
Working Reagent Ⅳ	80	80	80	0

Mix thoroughly and incubate at room temperature for 15 min				
Reagent Ⅴ	40	40	40	0
Reagent Ⅵ	40	40	40	40
Reagent Ⅴ	0	0	0	40
Working Reagent Ⅳ	0	0	0	80
Mix thoroughly on a vortex oscillator for 1 min, then transfer 200 μL of the supernatant to a 96-well plate. Measure the absorbance at 540 nm, the readings are recorded as A_{Blank}, $A_{Standard}$, A_{Test} and $A_{Control}$, respectively, calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: The Blank Tube and Standard Tube only need to be done once or twice, each sample assay should include a corresponding control. It is recommended to select 2-3 samples with expected significant differences for a preliminary test. If ΔA_{Test} is less than 0.02, the sample amount can be appropriately increased. If ΔA_{Test} exceeds 0.4, the supernatant can be further diluted with deionized water. Multiply the final result by the dilution factor, or reduce the amount of sample used for extraction.

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.

Calculation of Ach Content:

(1) Based on whole blood volume

Ach (μg/mL)=$\Delta A_{Test}\div\Delta A_{Standard}\times C_{Standard}\times 4$**=$\Delta A_{Test}\div\Delta A_{Standard}\times 200$**

(2) Based on serum volume

Ach (μg/mL)=$\Delta A_{Test}\div\Delta A_{Standard}\times C_{Standard}\times 2.25$**=$\Delta A_{Test}\div\Delta A_{Standard}\times 112.5$**

$C_{Standard}$: Final concentration of the standard, 50 μg/mL. 4: Initial dilution factor of whole blood. 2.25: Initial dilution of serum.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

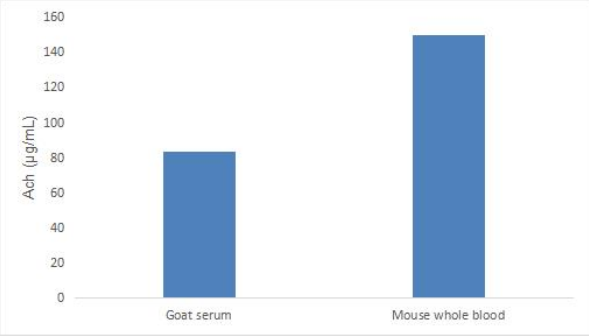

Figure 1. This kit is used to determine the Ach content in Goat serum and Mouse whole blood

Recommended Products

Catalog No.	Product Name
KTB1200	CheKine™ Micro Protein Carbonyl Assay Kit
KTB1551	CheKine™ Micro Non-protein Sulfhydryl Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.