

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

Acetylcholine (ACh) Activity Assay Kit

Catalog No.: BC00134

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Acetylcholine (ACh) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Whole blood, serum, plasma
Assay Type	Quantitative
Detection Instrument	Microplate reader (optimal detection wavelength 550 nm)

Product Introduction

Acetylcholine (ACh) is one of the earliest studied neurotransmitters. It is an excitatory neurotransmitter in many peripheral nerves, such as motor nerves, preganglionic fibers of the autonomic nervous system, and postganglionic fibers of the parasympathetic nervous system. Measuring the acetylcholine level in the blood is of certain significance in determining autonomic nerve function and its role in the pathogenesis of certain diseases, such as bronchial asthma, chronic bronchitis, eczema, and peptic ulcer disease.

Principle

Acetylcholine reacts with a substrate to produce a brown compound through a colorimetric reaction. The absorbance of the compound is measured, and the intensity of the color is directly proportional to the concentration of acetylcholine.

Components

Product Name	Name	Size	Storage
Reagent 1	Working solution	Powder A ×1 vial, Powder A solvent 3.5 mL ×1 bottle; Solution B 3.5 mL ×1 bottle.	2~8°C

Reagent 2	Terminator	6 mL	2~8°C
Reagent 3	Color developer	6 mL	2~8°C, away from light
Reagent 4	Clarifying agent	10 mL	RT
Reagent 5	Ach Standard	3 vials of powder, 6 mL of diluent	2~8°C
Reagent 6	Extract	3 mL	2~8°C
Reagent 7	Hemolytic agents	10 mL	2~8°C
Reagent 8	Precipitator	10 mL	2~8°C
Consumables 1	Microplate	1 plate	RT
Consumables 2	Plate Sealer	2 pieces	RT

Storage

The kit has a shelf life of 3 months.

Preparation before the experiment

Sample processing

1. Whole blood sample: Take 100 μ L of whole blood, add 175 μ L of Reagent 7 (hemolysin), mix well in a vortex mixer and extract for 3 minutes, add 25 μ L of Reagent 6 (extractant), mix well, let stand for 2 minutes, add 100 μ L of Reagent 8 (precipitant), centrifuge at 3500 rpm for 10 minutes, and take the supernatant for testing.
2. Serum (plasma): Take 200 μ L of serum (plasma) and add 50 μ L of reagent 6 (extraction agent), mix well, let stand for 2 minutes, add 200 μ L of reagent 8 (precipitant), centrifuge at 3500 rpm for 15 minutes, and take the supernatant for testing.

Preparation of the reagent kit

1. Preparation of working solution for reagent 1: Before use, add one vial of powder A to one bottle of powder A solvent to prepare solution A. Mix solution A and solution B in a ratio of

1:1 before use. Prepare the required amount.

2. Reagent 4 will solidify at low temperatures. Before use, dissolve it completely in a water bath at 37°C for at least 5 minutes until it is clear and transparent.
3. Preparation of 10 mg/mL standard application solution: Immediately before use, add 0.25 mL of standard diluent to one vial of standard powder to prepare a 10 mg/mL standard application solution. Mix well and dissolve completely (this solution can be used to prepare a standard curve). Then, add 10 µL of the 10 mg/mL standard application solution to 990 µL of standard diluent, mix well, and you will have a 100 µg/mL standard application solution. Store at 4°C for no more than 3 days.

4. Preparation of standards:

Dilute 10 mg/mL of the standard working solution with standard diluent to prepare concentrations of 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.5 mg/mL, 0.3125 mg/mL, and 0 mg/mL, respectively, according to the table below:

5. Operation table

Pipe number	1	2	3	4	5	6	7	8
Standard concentration (mg/mL)	0	0.313	0.5	0.625	1.25	2.5	5	10
Ach standard sample size (µL)	--	10	10	10	10	10	10	10
Double-distilled water (µL)	100	90	90	90	90	90	90	90
Reagent 1 (µL)	100	100	100	100	100	100	100	100
Mix well and let stand at room temperature for 15 minutes.								
Reagent 2 (µL)	50	50	50	50	50	50	50	50
Reagent 3 (µL)	50	50	50	50	50	50	50	50
Reagent 4 (µL)	30	30	30	30	30	30	30	30
Mix well, take 0.2 mL of the supernatant and add it to a 96-well plate. Measure the OD value (A) of each well at 550 nm using a microplate reader.								

Operation process

Reagents(μL)	Blank tube	Standard pipe	Test tube	Control tube
Double-distilled water	100	--	--	150
100 μg/mL Ach standard	--	100	--	--
Sample to be tested	--	--	100	100
Reagent 1	100	100	100	--
Mix well and let stand at room temperature for 15 minutes.				
Reagent 2	50	50	50	--
Reagent 3	50	50	50	50
Reagent 4	30	30	30	30
Mix well, centrifuge at 3500 rpm for 10 minutes, and add 0.2 mL of the supernatant to a 96-well plate. Measure the OD value (A) of each well at 550 nm using a microplate reader.				

Result calculation

$$\text{ACH Content} \left(\frac{\mu\text{g}}{\text{mL}} \right) = \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times N$$

Annotation

C standard : standard concentration, 100 μg/mL;d: Cuvette path length (cm), 1cm;

N : Sample dilution factor before testing, including pretreatment dilution factor (i.e., 4-fold dilution of whole blood, 2.25-fold dilution of serum).

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.