Pioneer Research Anahita Company
No.157
Danesh street
Technology Park Pardis
Tehran/ Iran

DATA SHEET
Versions: 01
Revision date: 25/11/2023

1. Identification

Product name: AChE Activity Assay Kit

Reactions: 100 rxns

Cat. No.: PRA-SOD

2. Description

Acetylcholinesterase (AChE) is a cholinergic enzyme primarily located in postsynaptic neuromuscular junctions, particularly in muscles and nerves. Its primary function is the rapid hydrolysis or breakdown of acetylcholine, a natural neurotransmitter, into acetic acid and choline. This enzymatic activity is crucial for terminating the action of acetylcholine and regulating neural signaling. It's worth noting that in addition to its presence in neuromuscular junctions, AChE is also found in serum and red blood cells, suggesting its involvement in various physiological processes beyond synaptic transmission. The widespread distribution of acetylcholinesterase highlights its importance in maintaining proper cholinergic neurotransmission and overall physiological balance.

3. Kit Contents

Component	Cat. no
Acetylcholinesterase reagent	PRA-AChER
Standard	PRA-AChES

4. Storage specifications

AChE Activity Assay Kit components can be stored at room temperature.

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5. Applications

Assay of acetylcholinesterase activity plays an important role in diagnostic, detection of pesticides and nerve agents, in vitro characterization of toxins and drugs including potential treatments for Alzheimer's disease.

6. Assay Procedure

Preparation of Solutions:

Acetylcholinesterase Reagent:

To prepare the acetylcholinesterase reagent, mix 3 ml of 96% ethanol with 7 ml of distilled water and add it to the container of the acetylcholinesterase reagent. Mix well. If the reagent does not dissolve well (due to the difference in the quality of ethanol), try to dissolve the reagent using an ultrasonic bath or a sonicator probe. If the mentioned devices are not available, vortex well (at least 3 minutes).

The prepared reagent is stable for 72 hours in a dark place.

Sample Preparation:

- For total acetylcholinesterase activity: Dissolve 10 µl of whole blood containing anticoagulant with 8 ml of distilled water. After vigorous mixing, incubate for 5 minutes at room temperature.

- For acetylcholinesterase activity in pure RBC: Wash 1 cc of blood with 10 cc of physiological serum three times. Then, dissolve 100 µl of the washed blood with 8 ml of distilled water. Mix vigorously and incubate for 5 minutes at room temperature. Dilute the blood at a ratio of 1/20 with PBS buffer.

- For tissue sample acetylcholinesterase activity: Homogenize 50 mg of the tissue in 2 ml of PBS buffer, then centrifuge for 5 minutes at 5000 RPM and use the supernatant for testing.

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Protocol:

1. Keep the kit components at room temperature for 20 minutes before starting the test.

2. Add 100 µl of the sample (prepared whole blood, washed, or tissue supernatant) to a clean, sterile microtube.

3. Add 100, 50, 25, 12, 6, and 3 µl of the standard to separate vials (named as 1 to 6 with concentrations 72 U/ml, 36 U/ml, 18 U/ml, 9 U/ml, 4.5 U/ml, and 2.25 U/ml, respectively).

4. Add 100 µl of distilled water to the blank vial.

5. Add 100 µl of acetylcholinesterase reagent to all wells and incubate for 40 minutes at 37°C.

6. Add 100 µl of the colored solution to the ELISA plates and measure at a wavelength of 560 nm.

- First, decrease the OD values of the blank from the samples and standards.
- Draw a graph and obtain the values of the samples based on the graph.
- Note: Normal values of acetylcholinesterase activity are 18-8 U/ml.

7. Safety

- The solutions used in this kit are dangerous for human tissue.
- **Work with gloves and protective eye wear.
- In case of contact with skin, eyes, etc., wash with plenty of water.
- Seek medical attention promptly for additional treatment.

8. Quality Certifications

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9. Further information

This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

10. Other Kits

1. Total Antioxidant Activity Test Kit (FRAP)
2. Catalase activity testing kit
3. Kit to check the amount of NO
4. FRAP Assay test kit
5. Paraoxonase-1 activity testing kit
6. Protein carbonyl testing kit

NOTE

All products have been produced by Karmania Pars Gene company in Iran.