antibody and ELISA experts

ACETYLCHOLINESTERASE (ACHE) ACTIVITY ASSAY KIT

for measuring AChE activity in

different types of biological samples

This package insert must be read in its entirety before using this product.
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Instruction Manual

Acetylcholinesterase (AChE) Activity Assay Kit Catalog No.: AR4001 and AR4001-1

INTRODUCTION

Acetylcholinesterase (AChE) is known to be one of the most important enzymes involved in neural responsiveness and hydrolyzes the neurotransmitter acetylcholine to acetate and choline. It is also known as RBC cholinesterase, mainly present in the blood and at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate the synaptic transmission. AChE has a very high catalytic activity and each molecule of AChE degrades about 5,000 molecules of acetylcholine per second. It is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen. Changes in AChE activity may result from exposure to certain compounds/drugs, which act as cholinesterase inhibitors. AChE inhibitors are among the key drugs approved for Alzheimer's disease (AD), dementia and myasthenia gravis. 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. Novel drugs for Alzheimer's disease or antidotal therapy are tested by *in vitro* methods when AChE is implicated in the treatment process.

Our Acetylcholinesterase Assay Kit provides a simple, sensitive and direct procedure for measuring AChE activity in different types of biological samples such as blood, serum, and plasma. The assay is an optimized and improved version of the Ellman method in which thiocholine, produced by AChE, reacts with 5,5'-dithiobis (2-nitrobenzoic acid) and forms yellow color, which is proportional to the AChE activity present in the sample and can be measured at 410-412 nm. The kit is robust and can be used for continuously monitoring AChE activities and has a linear range of 10–600 units/L of AChE activity. It detects as little as 0.1 mU AChE in a 100 µl assay volume (1 mU/ml) and one unit of AChE is the amount of enzyme that catalyzes the production of 1.0 mmole of thiocholine per minute at pH 7.5 at room temperature.

Acetylthiocholine $\xrightarrow{\text{AChE}}$ Thiocholine + Acetate

Thiocholine + Dithiobisnitrobenzoate (DTNB) ⟶ Yellow Color (Read at 412 nm)
[5-thio-2-nitrobenzoate + oxidized thiocholineiodide]

Key Features

- **Sensitive**- detect as low as 0.1 mU of acetylcholinesterase in the sample
- **Detection Range**- 10-600 U/L AChE activity
- **Broad Application-** Can be used to measure acetylcholinesterase activity in different biological samples.
- **High-throughput**- Can be easily adapted to automation as a high-throughput 96-well plate for large number of samples.
- **Convenient-** Formulated to have minimal hands-on time. The method involves adding a single working reagent, and reading the optical density at 2 min and 10 min at room temperature.
- **Non-Hazardous-** No special requirements for waste treatment

Items Supplied:

Item Name	Cat. No. AR4001	Cat. No. AR4001-1	Storage Condition*
DTNB Reagent [20X]	1 ml	2 ml	-20°C
Assay Buffer	25 ml	50 ml	-20°C
Acetylthiocholine (ATC) [20X]	1 ml	2 ml	-20°C
Acetylcholinesterase (AChE) Standard	1 Vial (5 Units)	1 Vial (10 Units)	-20°C
0.1% BSA Solution	250 µl	500 µl	-20°C

**The kit is shipped in blue ice and upon receipt, store it at -20°C protected from light exposure and it is stable for 6 months, if stored and used as recommended.*

Additional Items Needed:

- 96 or 384 Well Clear Bottom Microplate
- Microplate reader
- Molecular Grade Water or ddH_2O

Summary of the AChE Assay Steps

Prepare AchE Reaction Mixture

⇓

Add AChE standards or AChE test samples to the Reaction Mixture

⇓

Incubate at room temperature for 10-30 minutes

⇓

Read and Monitor the Absorbance at 405-415 nm

Sample Preparation

Blood samples should be diluted 40-fold in the Assay Buffer. Tissue or cell lysates can be prepared by briefly sonicating or by homogenization in 100 mM Tris buffer, pH 7.0, followed by centrifugation at 14,000 rpm for 5 minutes and use clear supernatant for AChE assay. Best results are obtained when lysates are freshly prepared. If this is not feasible, lysates should be stored at 2–8 °C and used within 24 hours.

Preparation of Solutions & Assay Reaction Setup

1. **Preparation of Acetylthiocholine Reaction Mix**
   Add 250 µl of DTNB Reagent (20X) and 250 µl of Acetylthiocholine (ATCI) Substrate (20X) into 4.5 ml of Assay Buffer to make a total volume of 5 ml Acetylthiocholine Reaction Mix. This Acetylthiocholine Reaction Mix will be good for ONE 96-well plate. Keep it protected from the light.

2. **Preparation of Acetylcholinesterase Standard Solution**:
   Add 100 µl of the supplied 0.1% BSA into the vial of Acetylcholinesterase (AChE) Standard to make a 50 Units/ml (or 5 Units/100µl) Acetylcholinesterase Standard solution, gently tab the tube with finger to mix completely (DO NOT SHAKE). NOTE: Diluted acetylcholinesterase standard solution is **not very stable** and should be used on the same day. If you want to use it at a later time, immediately freeze it at -20°C, however, the AChE activity will drop within a few days.

3. **Preparation of serial dilutions of Acetylcholinesterase (AChE) Standard (0 to 1000 mU/ml):**
   Add 20 µl of 50 U/ml acetylcholinesterase standard solution to 980 µl of Assay Buffer to generate 1000 mU/ml acetylcholinesterase standard solution (AS7). Then take 1000 mU/ml acetylcholinesterase standard and perform 1:3 serial dilutions to get remaining serial dilutions of acetylcholinesterase standard (AS6 - AS1).

Table-1:

Tube No.	**Assay Buffer** (µl)	**AChE Standard** (µl)	**AChE** (mU/ml)
B (Blank)	150 µl	0 µl	0
1	980 µl	20 µl of 50 U/ml Tube	1000
2	150 µl	75 µl of Tube 1	333.33
3	150 µl	75 µl of Tube 2	111.11
4	150 µl	75 µl of Tube 3	37.04
5	150 µl	75 µl of Tube 4	12.35
6	150 µl	75 µl of Tube 5	4.12
7	150 µl	75 µl of Tube 6	1.37

Details of AChE Serial Dilutions

I. First create a tube for blank using 150 µl of Assay Buffer and label as Tube B.

II. Pipette 980 µl Assay Buffer and add 20 µl of 50 U/ml Acetylcholinesterase standard into it (Tube 1). Mix well gently (DO Not SHAKE) and avoid generating bubbles.

III. Pipette 75 µl of Tube 1 into 150 µl of Assay buffer to create Tube 2. Mix well gently (DO NOT SHAKE) and avoid generating bubbles.

IV. Pipette 75 µl of Tube 2 into 150 µl of Assay buffer to create Tube 3. Mix well gently (DO Not SHAKE) and void generating bubbles.

V. Pipette 75 µl of Tube 3 into 150 µl of Assay buffer to create Tube 4. Mix well gently (DO Not SHAKE) and avoid generating bubbles.

VI. Pipette 75 µl of dilution 4 (from step 4) into 150 µl of Assay buffer to create dilution 5. Mix well gently (DO Not SHAKE) and avoid generating bubbles.

VII. Pipette 75 µl of dilution 5 (from step 5) into 150 µl of Assay buffer to create dilution 6. Mix well gently (DO Not SHAKE) and avoid generating bubbles.

VIII. Pipette 75 µl of dilution 6 (from step 6) into 150 µl of Assay buffer to create dilution 7. Mix well gently (DO Not SHAKE) and avoid generating bubbles.

NOTE: The Tube No. 7 would have an extra 75 µl volume as compared from other tubes.

Assay Protocol Steps:

Important- Thaw all the kit components to room temperature before starting the experiment.
Addition of Working Reagent should be quick and mixing should be brief but thorough as this assay is based on an enzyme-catalyzed kinetic reaction. Use of a multichannel pipette is recommended.

1. Layout of acetylcholinesterase standards and test samples in a clear bottom **96-well microplate**.
   AS= Acetylcholinesterase Standards (AS1-AS7, 1 to 1000 mU/mL); BL=Blank Control; TS=Test Samples.
   Table-2:

BL	BL	TS	TS		….	….					
AS1	AS1	….	….		….	….					
AS2	AS2										
AS3	AS3										
AS4	AS4										
AS5	AS5										
AS6	AS6										
AS7	AS7										

(***BL****= Blank Control,* ***AS****=* Acetylcholinesterase *Standards*, ***TS****= Test Samples)*

2. Reagent content & volume for each well.
   Table-3:

Well No.	**Volume**	**Reagent**
AS1-AS7	50 µl	1 to 1000 mU/ml Serial Dilutions
BL	50 µl	Assay Buffer
TS	50 µl	Test Sample

3. Add 50 µl of **Acetylthiocholine Reaction Mix** to each well of the Acetylcholinesterase Standard, Blank Control, and Test Samples to make the total acetylcholinesterase assay volume of 100 µl/well.
   [Note: For a 384-well plate, add 25 µl of sample and 25 µl of acetylthiocholine reaction mix in each well.]
4. Incubate the reaction tubes for 10-30 minutes at room temperature, protected from light.

5. Monitor the absorbance increase, using a colorimetric microplate reader at 405-415 nm (max abs at 412 nm)
   ***Note**: The absorbance background increases with time, thus it is important to subtract the absorbance intensity value of the blank wells for each data point.*

Data Analysis

The absorbance in blank wells (containing assay buffer only) is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the AChE standards readings to obtain a standard curve and equation or OD change per minute, that can be used to calculate Acetylcholinesterase activity in the test sample(s). An example acetylcholinesterase standard curve is shown in Figure-1.

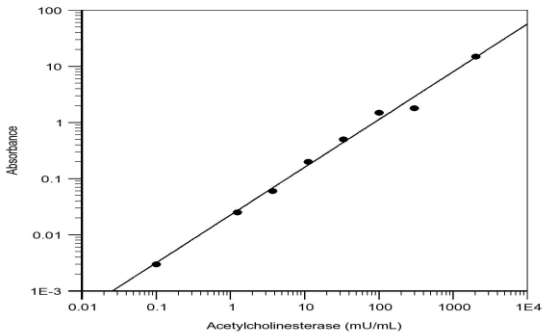

Figure-1: Acetylcholinesterase standard, Abs measured 412nm, using a clear bottom 96-well plate with our Acetylcholinesterase Assay Kit (Colorimetric) with a microplate reader. As low as 0.1 mU/well of acetylcholinesterase can be detected with 30 minutes incubation (n=3).

Troubleshooting Guide:

Problem	Possible Cause	Solution
Assay not working	Assay buffer is at wrong temperature	Assay buffer **must not be** chilled and needs to be at Room Temp
	Omission of step(s) in the assay protocol	Re-read the protocol and follow it exactly
	Plate read at incorrect wavelength	Make sure for appropriate reader and filter settings
	Unsuitable microtiter plate for assay	Clear plate to use for this colorimetric assay
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings as per the assay protocol
	Unsuitable sample type	Use recommended samples types as listed in the protocol
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

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