ACHE Colorimetric Assay Kit

82281
96 reactions

Description

The ACHE Colorimetric Assay Kit is designed to measure the activity of ACHE (acetylcholinesterase) for screening and profiling applications. The ACHE colorimetric reaction is based on a modified Ellman's method, using the alternative substrate acetylthiocholine iodide (ATCI) and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) to quantify amount of thiocholine produced from the hydrolysis of ATCI by ACHE. This assay kit comes in a convenient 96-well format, with purified ACHE enzyme (amino acids 32-614), ATCI, DTNB and assay buffer for 100 enzyme reactions. The absorption intensity of DTNB adduct (λ=410 nm) is used to measure the amount of thiocholine product generated, which is proportional to ACHE activity.

Background

ACHE (acetylcholinesterase), also known as AChase or acetylhydrolase, is known to hydrolyze acetylcholine (ACh), a naturally occurring neurotransmitter, into acetic acid and choline. It is a highly effective hydrolase, with an activity near the limit of substrate diffusion. It can be found in motor and sensory neurons, and it is involved in the termination of impulse transmission, by being located on the post-synaptic membrane and hydrolyzing Ach. Ach can be taken up by and used to synthesize acetyl-Coa again in a reaction catalyzed by choline acetyltransferase. ACHE can exist in different molecular forms, which have different expression patterns. It has been known for many years that AD (Alzheimer's disease) patients have an abnormal distribution of these molecular forms, with an increase in the light forms versus G4 molecules. P-tau can lead to higher expression of T-ACHE, and ACHE may play a role in formation of beta-amyloid plaques. Inhibitors of ACHE result in high concentrations of acetyl-COA and continuous signaling, and if they are irreversible and can lead to muscular paralysis, convulsions, difficulty breathing and asphyxiation. A well-known class of such irreversible inhibitors are organophosphates, that have been used as nerve gases and insecticides. Reversible inhibitors have been approved by the FDA and used to attempt to improve neurological disorders, such as Alzheimer's disease, myasthenia gravis and Lewy body dementia.

Application(s)

- Screen molecules that inhibit ACHE in drug discovery high-throughput screening (HTS) applications.
- Determine ACHE inhibitor IC_{50} values.
- Perform ACHE activity real-time kinetics analysis.

Supplied Materials

Catalog #	Name	Amount	Storage
11004	ACHE, His-Tag*	>1 µg	-80°C
	DTNB (Lyophilized)	100 reactions	-80°C
	Acetylthiocholine Iodide (ATCI) (Lyophilized)	100 reactions	-80°C
79311	3x Immuno Buffer	4 ml	-80°C
79963	Clear, nonbinding Corning, 96-well microtiter plate		Room Temp

** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Spectrophotometer capable of measuring absorbance at l=410-415 nm

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This assay kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control", and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using chlorpyrifos-oxon as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Thaw **ACHE, DTNB, and Acetylthiocholine Iodide (ATCI)** on ice. Briefly spin the tubes to recover their full content.

2. Prepare 1x Immuno Buffer by diluting 3x Immuno Buffer 3-fold with distilled water.

3. Resuspend lyophilized DTNB with 550 µl of distilled water.

4. Resuspend lyophilized Acetylthiocholine Iodide (ATCI) with 70 µl of distilled water.

5. Dilute ACHE with 1x Immuno Buffer to 0.01 ng/µl (20 µl/well).

6. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x Immuno Buffer, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x Immuno Buffer (Diluent Solution).

   OR

   6.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Immuno Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Immuno Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Immuno Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

7. Dilute the resuspended DTNB 4-fold with 1x Immuno Buffer.
8. Add 20 µl of diluted DTNB to each well.
9. Add 5 µl of Test Inhibitor solution to each well designated "Test Inhibitor".
10. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank".
11. Add 20 µl of diluted ACHE to each well designated "Positive Control" and "Test Inhibitor".
12. Add 20 µl of 1x Immuno Buffer to the "Blank" wells.
13. Preincubate reaction at Room Temperature (RT) for 30 minutes.
14. Dilute Acetylthiocholine Iodide (ACTI) 8-fold with 1x Immuno Buffer.
15. Add 5 µl of diluted Acetylthiocholine Iodide (ACTI) to each well.

Component	Test Inhibitor	Blank	Positive Control
Diluted DTNB	20 µl	20 µl	20 µl
Test Inhibitor	5 µl	–	–
Diluent Solution	–	5 µl	5 µl
ACHE (0.01 ng/µl)	20 µl	-	20 µl
1x Immuno Buffer	-	20 µl	-
After 30 minutes of pre-incubation			
Diluted Acetylthiocholine iodide (ACTI)	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**

16. Incubate at RT for 30 minutes.
17. Read absorbance at λ= 410 nm.
18. The "Blank" value is subtracted from all other readings.

Example Results

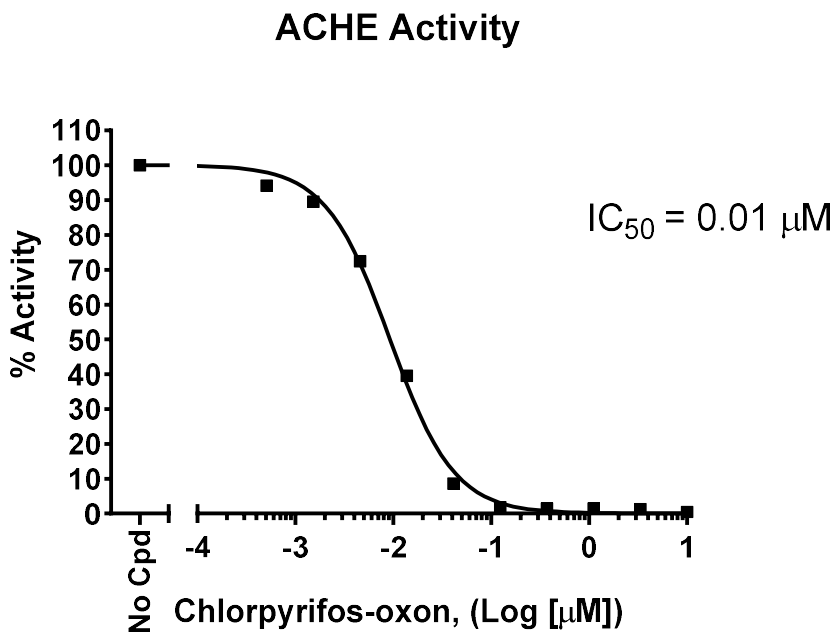

Figure 1: Inhibition of ACHE by Chlorpyrifos-oxon.
ACHE activity was measured in presence of increasing concentrations of Chlorpyrifos-oxon (Medchem Express #HY-136610). Results are expressed as percent activity, in which absence of inhibitor is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Garcia-Ayllon M., *et al.*, 2011 *Front Mol Neurosci* 4:22.

Related Products

Products	*Catalog #*	*Size*
Anti-β-amyloid, Plaque Neutralizing Antibody	79468	100 µg
GFRAL, Fc Fusion, Avi-Tag Recombinant	101012	100 µg/1 mg
Human Superoxide Dismutase Recombinant	90240-B	100 µg

Version 021624