SensoLyte® 520 Acetylcholinesterase Activity Assay Kit
Fluorimetric

Revision number: 1.3	Last updated: 12/5/2017
Catalog #	**AS-72242**
Kit Size	200 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect human acetylcholinesterase activity.
- ***Enhanced Value:*** It provides ample reagents to perform 200 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in 1 hour.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Acetylcholinesterase Substrate	100 µL
Component B	Acetylcholinesterase, Human Recombinant	1 µg/ml, 10 µL, 2 vials
Component C	Acetylthiocholin	100 µL
Component D	Assay Buffer	50 mL
Component E	Acetylcholinesterase Inhibitor	5 mM in DMSO, 20 µL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat bottom 96-well plate with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 nm.
- Plate cover: To prevent liquid evaporation during incubation step.

Storage and Handling

- Store kit Components A, C, D, and E at -20 °C. Store Component B at -80 °C.
- Protect Component A from light and from moisture.

Introduction

Acetylcholinesterase (AChE) is a fast acting serine hydrolase that belongs to the carboxylesterase enzyme family.[1-4] It hydrolyses acetylcholine into choline and acetic acid and is found in the synaptic cleft. It can be detected in many mammals and non-mammalian species.[1-4] Alzheimers and myasthenia gravis diseases are characterized by reduced acetylcholine. Thus, drugs that inhibit AChE were developed to reduce disease symptoms.[4] AChE can be inhibited by many natural substances such as alkaloids and synthetic chemicals such as organophosphorous compounds that are frequently used as pesticides.[2-4] Recently, it was demonstrated that natural compounds have variable inhibitory effect on AChE depending on the species.[5]

The SensoLyte® 520 Acetylcholinesterase Assay Kit is a homogeneous assay that can be used to detect the activity of enzyme and for screening of AChE inhibitors. Active AChE generates fluorescence that can be monitored at excitation/emission=490/520 nm. The long wavelength fluorescence of the substrate in this kit minimizes interference from autofluorescence of components in biological samples and test compounds, resulting in higher sensitivity. SensoLyte® 520 Acetylcholinesterase Assay Kit can detect as low as 8 pg/ml of active AChE.

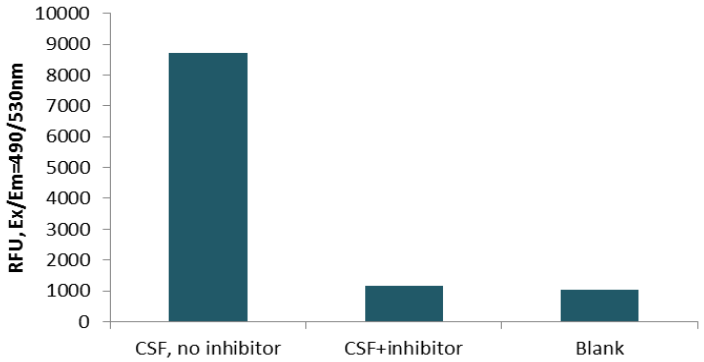

Figure 1. Measurement of AChE activity in human cerebrospinal fluid (CSF was diluted at 1:10 ratio with the Assay Buffer) in the presence or absence of AChE inhibitor (5μM neostigmine bromide) using SensoLyte® 520 Acetyl Cholinesterase Activity Assay Kit (Cat# AS-72242).

Protocol

1. Prepare working solutions.

Note 1: Allow all kit components to thaw before starting the experiment. Component B should be kept on ice.
Note 2: Briefly centrifuge Component B to completely recover enzyme.

1.1 AChE substrate solution: Prepare AChE substrate according to the Table 1.

50 μL of this diluted substrate is enough for one-well assay. For each experiment, prepare fresh substrate solution. If not using entire 96-well plate, prepare amount of substrate necessary for the experiment.

Table 1. AChE Substrate solution for one 96-well plate (100 assays).

Components	Volume
AChE substrate (Component A)	50 μL
Acetylthiocholin (Component C)	50 μL
Assay buffer (Component D)	4.9 mL
Total volume	5 mL

1.2 Recombinant human AChE diluent: Dilute the enzyme (Component B) 1:400 in assay buffer. This amount of enzyme is enough for a full 96-well plate. If not using the entire plate, adjust the amount of enzyme to be diluted accordingly.

Note: Dilute enzyme immediately before use. Store diluted enzyme on ice.

1.3 AChE Inhibitor: Dilute AChE Inhibitor, Neostigmine bromide (Component E), 1:100 with the Assay Buffer (Component D) to a final concentration of 50 µM. Suggested volume of the diluted inhibitor for one well in a 96-well plate is 10 µL that will result in 5 µM final inhibitor concentration.

2. Set up the enzymatic reaction.

2.1 Add test compounds and enzyme solution to the microplate wells. For one well of a 96-well plate, the suggested volume of AChE enzyme solution is 40 µL and 10 µL of test compound.

2.2 Establish the following control wells at the same time, as deemed necessary:

- Positive control contains AChE enzyme without test compound.
- Inhibitor control contains AChE and neostigmine bromide.
- Vehicle control contains enzyme and vehicle used in delivering test compound (e.g. DMSO, concentration not to exceed 1%).
- Test compound control contains assay buffer (Component D) and test compound. Some test compounds may inhibit developer and be fluorescent or interfere with the Ex/Em wavelengths and thereby giving false results.
- Substrate control contains assay buffer.

2.3 Using assay buffer (Component D) bring the total volume of all wells to 50 µL.

3. Run the enzymatic reaction.

3.1 Add 50 µL of AChE substrate solution into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix reagents completely by shaking the plate gently for no more than 30 sec.

3.2 Measure fluorescence signal:

For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 min. for 30 to 60 min.
For end-point reading: Incubate the reaction at room temperature for 60 min. Keep plate from direct light. Then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

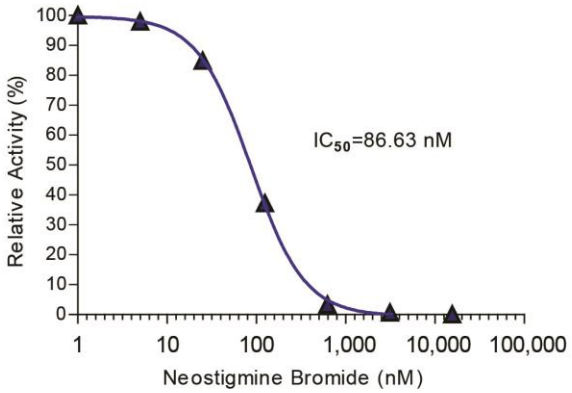

Figure 2. Neostigmine bromide inhibition of AChE activity measured with SensoLyte® 520 AChE Activity Assay Kit (Cat# AS-72242).

4. Data Analysis

4.1 The fluorescence reading from the substrate control well is used as the background fluorescence. This background reading should be subtracted from the readings of the other wells containing substrate. All fluorescence readings are expressed in relative fluorescence units (RFU).

References

1. Tong F., et.al., *Pesticide Biochem and Phys* **106** (2013): 79-84
2. Kozurkova M., et.al., *Pharmaceuticals* **4** (2011): 382-418
3. Lee S. R., et.al., *Mar Drugs* **12** (2014): 3560-3573
4. Pohanka M, *Int J Mol Sci* **15** (2014): 9809-9825
5. Ramli R. A., et.al., *Nat Prod Commun* **8** (2013): 695-698