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Product Information

Acetylcholinesterase Inhibitor Screening Kit

Catalog Number **MAK324**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Acetylcholinesterase (AChE), also known as RBC cholinesterase, is found primarily in the blood and neural synapses. AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. Inhibition of the enzyme leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission. AChE inhibition is an important target for the management of Alzheimer's disease, and AChE inhibitors are the most common drugs used for its management. In addition to Alzheimer's disease, AChE inhibitors have been useful in the diagnosis or treatment of diseases such as glaucoma, myasthenia gravis, and bladder distention.

The Acetylcholinesterase Inhibitor Screening kit is based on an improved Ellman method, in which thiocholine produced by the action of acetylcholinesterase forms a yellow color with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). The intensity of the product color, measured at 412 nm, is proportionate to the enzyme activity in the sample.

This kit can be readily automated on HTS liquid handling systems and is suitable for inhibitor screening and evaluation of acetylcholinesterase inhibitors.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.
Note: Neither the enzyme AChE nor control inhibitor is included in the kit.

Assay Buffer (pH 7.5) Catalog Number MAK324A	30 mL
Substrate (100 mM) Catalog Number MAK324B	500 µL
DTNB Catalog Number MAK324C	60 µL

Reagents and Equipment Required but Not Provided.

- Purified AChE (Catalog Number C3389)
- If desired, a control AChE inhibitor (e.g., Physostigmine)
- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96 well flat bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped at room temperature. Store the substrate and DTNB at –20 °C and all other components at room temperature upon receiving.

Procedures
This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Reaction Mix should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended.
Note: Neither AChE nor a control inhibitor is included in the kit.

Use ultrapure water for the preparation of all reagents and samples.

The following protocol is optimized for AChE from *E. electricus*. If another species is being analyzed, it is recommended that one experimentally determine the K_M value and then adjust the volume of substrate in the Reaction Mix so that the final concentration of the substrate in the 200 µL reaction is near the K_M.

Reagent Preparation
Equilibrate all components to desired reaction temperature.

Reaction Mix
For each well of reaction, prepare Reaction Mix by mixing into a clean tube:

154 µL of Assay Buffer
1 µL of Substrate
0.5 µL of DNTB

Note: Volume of Substrate can be adjusted if species other than *E. electricus* is being analyzed.

The Reaction Mix should be prepared freshly and used within 30 minutes.

Sample Preparation
Dissolve the test compounds (i.e., inhibitors) in solvent of choice. If using DMSO, it is prudent to first test the tolerance of DMSO by the enzyme to be inhibited. For AChE from *E. electricus*, the DMSO concentration of the 5 µL of test compounds added to the reaction should be 40% (v/v) DMSO or less.

Reference Enzyme Preparation
Prepare purified AChE to a concentration of 400 Units/L using Assay Buffer.

96 well Plate Reaction
1. Transfer 45 µL of AChE Reference Enzyme into separate wells.
2. Transfer 45 µL of Assay Buffer into one well. This is the Control (No Enzyme) well which can be used as a 100% inhibition control.
3. To the Control (No Enzyme) well and one well containing AChE Reference Enzyme (No Inhibitor Control), add 5 µL of the solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 40% (v/v) DMSO, add 5 µL of 40% (v/v) DMSO to these wells.
4. To the remainder of the wells containing AChE, add 5 µL of the test compounds. Incubate the plate for 15 minutes.
5. Add 150 µL of Reaction Mix to each sample, Control (No Enzyme), and No Inhibitor Control wells. Tap plate to mix.
6. Measure the absorbance at 412 nm (A_{412}) at 0 minutes and at 10 minutes.

384 well Plate Reaction
The procedure is similar to the 96 well plate assay, except that 18 µL of AChE Reference Enzyme is incubated with 2 µL of test compound (inhibitor) and then mixed with 30 µL of Reaction Mix (prepared by adding 32 µL of Assay Buffer, 0.25 µL of Substrate, and 0.125 µL of DTNB).

Results
Acetylcholinesterase activity is calculated as follows:

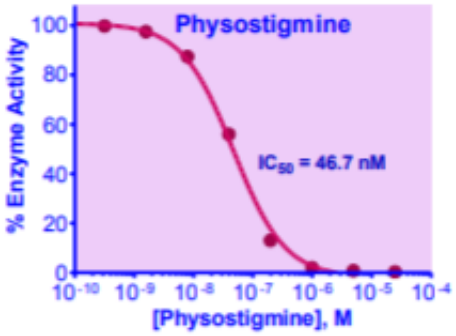
% Inhibition = $(1 - \Delta A_{\text{Test Cpd}} / \Delta A_{\text{No Inhibitor}}) \times 100\%$

where:

$\Delta A_{\text{Test Cpd}}$ = the A_{412} value of a test compound well at 0 minutes subtracted from the A_{412} value of the same well at 10 minutes

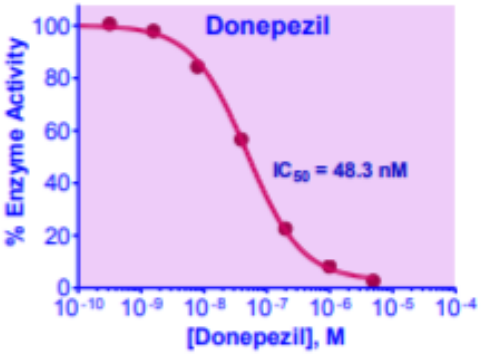
$\Delta A_{\text{No Inhibitor}}$ = the A_{412} value of the No Inhibitor Control well at 0 minutes subtracted from the A_{412} value of the No Inhibitor Control well at 10 minutes.

Figure 1.
Physostigmine titrations



AChE from *E. electricus* was incubated with various concentrations of Physostigmine. Each concentration of inhibitor contained 20% (v/v) DMSO (final 0.5% (v/v) in 200 µL reaction).

Figure 2.
Donepezil titrations



AChE from *E. electricus* was incubated with various concentrations of Donepezil. Each concentration of inhibitor contained 20% (v/v) DMSO (final 0.5% (v/v) in 200 µL reaction).

References

1. Magnottl, R.A. et al. Measurement of Acetylcholinesterase in Erythrocytes in the Field. Clin. Chem., **33/10**, 1731-1735 (1987).
2. Kovarik, Z. et al. Acetylcholinesterase active centre and gorge conformations analysed by combinatorial mutations and enantiomeric phosphonates. Biochem. J., **373**, 33–40 (2003).
3. Ordentlich, A. et al. The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions with Selected Organophosphate Inhibitors. J. Biol. Chem., **271 (20)**, 11953–11962 (1996).

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