Acetylcholinesterase Inhibitor Screening Kit (Colorimetric)

Acetylcholinesterase Inhibitor Screening Kit (Colorimetric) is a screening kit for inhibitor screening and evaluation of acetylcholinesterase inhibitors.

Catalog number: ARG82128

Package: 100 tests

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INTRODUCTION

Acetylcholinesterase inhibitors (AChEIs) also often called cholinesterase inhibitors, inhibit the enzyme acetylcholinesterase from breaking down the neurotransmitter acetylcholine into choline and acetate, thereby increasing both the level and duration of action of acetylcholine in the central nervous system, autonomic ganglia and neuromuscular junctions, which are rich in acetylcholine receptors. Acetylcholinesterase inhibitors are one of two types of cholinesterase inhibitors; the other being butyryl-cholinesterase inhibitors. Acetylcholinesterase is the primary member of the cholinesterase enzyme family.

Acetylcholinesterase inhibitors are classified as reversible, irreversible, or quasi-irreversible (also called pseudo-irreversible) [Provide by Wikipedia: Acetylcholinesterase inhibitor]

PRINCIPLE OF THE ASSAY

This Acetylcholinesterase inhibitor screening Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Acetylcholinesterase inhibitor in samples. The assay 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). The intensity of the product color, measured at O.D. 412 nm, is proportionate to the enzyme activity in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store the substrate and DTNB at-20°C and all other components at room temperature upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer, pH 7.5	30 mL	Room temperature
DTNB	60 µL	-20°C
Substrate (100 mM)	500 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 412 nm
- Centrifuge
- Clear flat-bottom 96 well plate
- Purified AChE (E.g. Sigma Aldrich cat# C3389) and if desired a control AChE inhibitor (E.g. Physostigmine, Santa Cruz Biotechnology cat# sc-202764).
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Sample preparation: Dilute purified AChE to 400 U/L using assay buffer. Dissolve the test compounds in solvent of choice. If using DMSO, it is prudent to first test the tolerance of DMSO by the enzyme of choice. For AChE from *E. electricus*, the DMSO concentration of the 5 µL of test compounds added to the reaction should be 40 v% DMSO or less.

REAGENT PREPARATION

- Working Reagent: The Working Reagent should be prepared freshly and used within 30 minutes. Each reaction well requires 154 µL of Assay Buffer, 1 µL of Substrate and 0.5 µL of DTNB. Take 150 µL Working Reagent to each well.

ASSAY PROCEDURE

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough.

Note: Neither AChE nor a control inhibitor is included in the kit.

1. Add **45 µL** of **AChE** into separate wells of a **96-well microplate**.
2. Add **45 µL** of **Assay Buffer** into one well of a **96-well microplate** as **No Enzyme Control** well which can be used as a 100% inhibition control.
3. To the **No Enzyme Control** well and **one well containing AChE** (**No Inhibitor Control**), add **5 µL** of **solvent** that the test compounds are dissolved in. For example, if the test compounds are dissolved in 40 v% DMSO, add 5 µL 40 v% DMSO to these wells.
4. Add **5 µL** of **test compounds** to the remainder wells and incubate the plate for **15 minutes**.
5. Add **150 µL** of **Working Reagent** to each sample, sample blank, and no-inhibitor control wells. Tap plate to mix.

   Note: Volume of Substrate can be adjusted if species other than *E. electricus* is being analyzed.
6. Read the absorbance at **O.D. 412 nm** at **0 minutes** (OD_0) and at **10 min** (OD_{10}).

CALCULATION OF RESULTS

1. Unit definition: one unit of enzyme catalyzes the production of 1 µmole of thiocholine per minute under the assay conditions (pH 7.5 and room temperature).
2. Acetylcholinesterase activity is calculated as follows:

   % Inhibition = [1 - (ΔOD_{Test} / $\Delta OD_{No\ Inhibitor}$)] x 100 %

   Note:

   - ΔOD_{Test}: O.D. 412 nm value ($OD_{10} - OD_0$) of test samples.
   - $\Delta OD_{No\ Inhibitor}$: O.D. 412 nm value ($OD_{10} - OD_0$) of No Inhibitor Control.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Acetylcholinesterase Inhibitor Screening Kit. One should use the data below for reference only. This data should not be used to interpret actual results.