



# **Acetylcholinesterase Inhibitor Screening Kit (Colorimetric)**

10/18

(Catalog # K197-100; 100 assays; Store at -20°C)

#### I. Introduction:

Acetylcholinesterase (AChE; EC 3.1.1.7) is the main cholinesterase enzyme in the nervous system. It is responsible for the hydrolysis of acetylcholine and some other choline esters that function as neurotransmitters. AChE is highly expressed in both the brain and the peripheral nervous system. It is especially enriched at neuromuscular junctions, where its activity serves to terminate synaptic transmission between motor neurons and skeletal muscles. Reversible inhibitors of AChE (such as donepezil, rivastigmine and galantamine) are commonly used in the treatment of Alzheimer's Disease and other neurodegenerative disorders. However, irreversible inhibitors of AChE tend to be extremely poisonous, causing muscular paralysis, convulsions, bronchial constriction and eventual death by asphyxia. BioVision's Acetylcholinesterase Inhibitor Screening Kit can be used to screen for potential inhibitors of AChE activity. It utilizes the ability of an active human AChE enzyme to hydrolyze the provided colorimetric substrate, generating a yellow chromophore that can be detected by measuring absorbance at 412 nm. In the presence of the potent reversible AChE inhibitor donepezil, enzyme activity is suppressed, preventing chromophore generation. The assay kit is adapted to a 96-well format and provides a rapid, simple and reliable test for high-throughput screening of AChE inhibitors.



#### II. Applications:

· Screening/characterizing Acetylcholinesterase inhibitors

#### III. Kit Contents:

Components	K197-100	Cap Code	Part Number
AChE Assay Buffer	50 ml	NM	K197-100-1
AChE Substrate	1 vial	Purple	K197-100-2
Acetylcholinesterase	1 vial	Green	K197-100-3
Probe Mix	1 vial	Red	K197-100-4
Donepezil (10 mM)	20 µl	Brown	K197-100-5

### IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Temperature-controlled plate reader

## V. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening.

- AChE Assay Buffer: Warm to room temperature before use. Store at 4 °C or -20 °C.
- AChE Substrate: Reconstitute in 100 μl of AChE Assay Buffer. Store at -20 °C, protected from light. Use within two months.
- Acetylcholinesterase: Reconstitute in 44 µl of AChE Assay Buffer. Aliquot and store at -20 °C. Use within two months.
- Probe Mix: Dissolve Probe Mix with 625 µl of AChE Assay Buffer. Store at -20 °C. Use within two months.
- Donepezil: Ready to use. Bring to room temperature before use.

## VI. Acetylcholinesterase Inhibitor Screening Protocol:

## 1. Screening Compounds, Inhibitor Control and Background Control Preparations:

Sample Compound [S]: Dissolve candidate inhibitors at 100X or higher concentration in an appropriate solvent. Further dilute to 20X with AChE Assay Buffer. Add 10 μl of diluted (20X) test inhibitors into designated wells of a clear, flat-bottom 96-well plate.

Enzyme Control (No Inhibitor) [EC] and Background Control [BC]: add 100 μl of Assay Buffer to designated well(s).

Inhibitor Control (Donepezil) [IC]: Prepare a 50-fold dilution of donepezil (to 0.2 mM) by adding 2 μl of the stock Donepezil (10 mM) solution to 98 μl of AChE Assay Buffer, mix well; further dilute the 0.2 mM solution to 20 μM by adding 4 μl of the 0.2 mM donepezil solution to 36 μl AChE Assay Buffer. Add 10 μl of the 20 μM donepezil working solution into designated well(s).

Additional wells with serial dilutions of the test inhibitors may be prepared at this time if desired. Each well should contain 10 µl of the test inhibitor at 20X the desired final concentration. Adjust the volume of each well to **100 µl/well** with AChE Assay Buffer.

	[S]	[IC]	[EC]	[BC]
Test Inhibitor	10 µl	-	=	-
20 µM Donepezil	-	10 µl	=	-
AChE Assav Buffer	=	=	100 ul	100 ul

**Note:** Organic solvents used to prepare test inhibitor stock solutions may impact AChE activity. We recommend preparing a parallel Solvent Control **[SC]** well with the same final concentration of solvent used to solubilize test inhibitor(s), in order to determine the effect of the solvent on AChE activity. If the activity obtained for the **[SC]** condition is significantly different from **[EC]**, use its values to determine the effect of tested compound.

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2. AChE Enzyme Solution Preparation: Prepare a 25-fold dilution of reconstituted Acetylcholinesterase (i.e. dilute 2 µl of Acetylcholinesterase with 48 µl of AChE Assay Buffer), mix well. Add 10 µl of Diluted Acetylcholinesterase to each well containing Sample Compounds [S], Inhibitor Control [IC], Solvent Control [SC] (if applicable) and Enzyme Control [EC]; Add 10 µl of AChE Assay Buffer to well containing Background Control [BC]. Adjust the volume of each well to 160 µl/well with AChE Assay Buffer. Mix well and incubate at room temperature for 10-15 min, protected from light.

Note: Discard unused, diluted Acetylcholinesterase Enzyme Solution after use.

3. Reaction Mix Preparation: Prepare a 12-fold dilution of AChE Substrate (i.e. dilute of 4 µl of AChE Substrate with 44 µl of AChE Assay Buffer). Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µl Reaction Mix containing:

	Reaction Mix
Diluted AChE Substrate	10 µl
Probe Mix	5 µl
AChE Assay Buffer	25 µl

Mix and add 40 µl Reaction Mix to Sample Compound [S], Inhibitor Control [IC], Enzyme Control [EC] (and Solvent Control [SC], if applicable) and Background Control [BC] wells. Mix well. The total reaction volume for each well will be 200 µl.

- 4. Measurement: Measure absorbance (OD at 412 nm) immediately in kinetic mode for 40 min at room temperature. Choose two time points (t<sub>1</sub> and t<sub>2</sub>) in the linear range of the plot and obtain the corresponding values for the absorbance (OD<sub>1</sub> and OD<sub>2</sub>).
- 5. Calculation: Calculate the slope for all Sample Compounds [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the net ΔOD<sub>412</sub> (OD<sub>2</sub>-OD<sub>1</sub>) values by the time Δt (t<sub>2</sub>-t<sub>1</sub>). Subtract the slope obtained for the Background Control reaction from the [S], [EC] and [SC] values. If the [SC] slope is significantly different than the [EC] value, use the [SC] value to calculate the effect of the sample compound.

$$\label{eq:Relative Inhibition} % \mbox{ Relative Inhibition} = \frac{\mbox{Slope of [EC]} - \mbox{Slope of [EC]}}{\mbox{Slope of [EC]}} \mbox{ X100}$$

% Relative Activity = 
$$\frac{\text{Slope of } [S]}{\text{Slope of } [EC]}$$
 X100

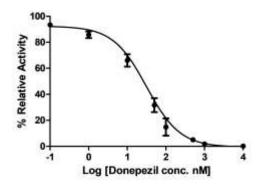


Figure: Inhibition of Acetylcholinesterase activity by Donepezil. IC<sub>50</sub> of Donepezil was calculated to be 31.5 ± 2.3 nM. Assay was carried out following the kit protocol.

## **RELATED PRODUCTS:**

Acetylcholinesterase Activity Colorimetric Assay Kit (K764-100)

Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615-100)

QuickDetect<sup>TM</sup> Acetylcholine (Ach) (Human) ELISA Kit (E4452) QuickDetect<sup>TM</sup> Acetylcholine (Ach) (Rat) ELISA Kit (E4454)

ACHE Antibody (CT) (6707)

Butyrylcholinesterase Activity Kit (K516-100)

Cholinesterase Activity Assay Kit (K975-100)

ACHE Antibody (NT) (6706)

QuickDetect<sup>™</sup> Acetylcholine (Ach) (Mouse) ELISA Kit (E4453)

BCHE Antibody (Center) (6724)

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