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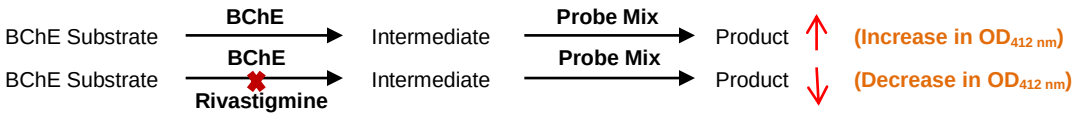
Butyrylcholinesterase Inhibitor Screening Kit (Colorimetric)

04/21

(Catalog # K2084-100; 100 assays; Store at -20 °C)

I. Introduction:

Butyrylcholinesterase, also known as BChE, BuChE, pseudocholinesterase, or plasma esterase is a cholinesterase enzyme that is mainly made in the liver and is present in blood plasma. It is a non-specific enzyme that hydrolyses various choline-based esters. It is also a physiological regulator of ghrelin signaling. BChE activity in plasma serves as a liver function test as both hypercholinesterasemia and hypocholinesterasemia indicate pathological processes. Additionally, reversible inhibitors of BChE such as rivastigmine, tacrine, and galantamine are commonly used in the treatment of Alzheimer's disease and other neurodegenerative disorders. However, irreversible inhibition of BChE tends to be extremely poisonous, causing muscular paralysis, convulsions, bronchial constriction, and eventually death by asphyxia. **BioVision's Butyrylcholinesterase Inhibitor Screening Kit** is a 96-well plate based, colorimetric assay to screen potential inhibitors of BChE. The kit utilizes the ability of active human BChE enzyme to hydrolyze the BChE substrate thereby generating a yellow colored product measured by absorbance at 412 nm. As the enzyme activity is inhibited in the presence of a potent, reversible BChE inhibitor, Rivastigmine, the color formation is inhibited. The assay is adapted for a 96-well plate format and provides a rapid, simple, and reliable test for high-throughput screening of BChE inhibitors.



II. Application:

- Screening or characterizing butyrylcholinesterase inhibitors

III. Kit Contents:

Components	K2084-100	Cap Code	Part Number
BChE Assay Buffer	50 ml	NM	K2084-100-1
BChE Substrate	100 µl	Blue	K2084-100-2
BChE Enzyme	1 vial	Green	K2084-100-3
BChE Probe Mix	1 vial	Red	K2084-100-4
Rivastigmine (40 mM)	200 µl	Amber	K2084-100-5

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Temperature-controlled plate reader
- 1X PBS

V. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **BChE Assay Buffer:** Store at 4 °C or -20 °C. Warm to room temperature (RT) before use.
- **BChE Substrate:** Divide into aliquots and store at -20 °C, protected from light. Bring to RT before use.
- **BChE Enzyme:** Reconstitute the vial in 55 µl of 1X PBS. Divide into aliquots and store at -20 °C. Use within two months. Keep on ice, while in use.
- **BChE Probe Mix:** Reconstitute the vial in 625 µl of BChE Assay Buffer. Store at -20 °C, protected from light. Use within two months.
- **Rivastigmine (40 mM):** Ready to use. Bring to RT before use.

VI. Butyrylcholinesterase Inhibitor Screening Protocol:

Screening Compounds, Inhibitor Control and Background Control Preparations:

1. Sample Compound [S]: Dissolve the test inhibitors at 100X or higher concentration in an appropriate solvent. Further dilute to 10X using BChE Assay Buffer. Add 10 µl of diluted (10X) test inhibitors into wells of a 96-well clear plate with flat bottom labeled as Sample **[S].** Add 10 µl of BChE Assay Buffer into the designated wells labeled as Enzyme Control **[EC]** and Background Control **[BC]** respectively. For the **Inhibitor Control [IC]** well, add 5 µl of the Rivastigmine (40 mM) into the designated well(s). Adjust the volume of all wells including Sample **[S],** Enzyme Control **[EC],** Background Control **[BC]** and Inhibitor Control **[IC]** to **10 µl/well** using BChE Assay Buffer.

	[S]	[IC]	[EC]	[BC]
Test Inhibitor	10 µl	-	-	-
40 mM Rivastigmine	-	5 µl	-	-
BChE Assay Buffer	-	5 µl	10 µl	10 µl

Notes:

a. Additional wells containing serial dilutions of the test inhibitors may be prepared, if desired. Each well should contain 10 µl of the test inhibitor at 10X the desired final concentration.

b. Organic solvents used to prepare the test inhibitor stock solutions may impact BChE activity. In order to determine the effect of solvent on the BChE activity, we recommend preparing a parallel Solvent Control **[SC]** well with the same final concentration of the solvent used to solubilize the test inhibitor(s). If the signal obtained in the Solvent Control **[SC]** well is significantly different from the **[EC]** well, use the signal for the SC well instead of the EC well.

2. BChE Enzyme Solution Preparation: Prepare a 10-fold dilution of the reconstituted BChE Enzyme by adding 5 µl of BChE Enzyme to 45 µl of BChE Assay Buffer and mix well. Add 5 µl of diluted BChE Enzyme to each well containing Sample **[S]**, Inhibitor Control **[IC]**, Solvent Control **[SC]** and Enzyme Control **[EC].** Add 5 µl of BChE Assay Buffer to the Background Control **[BC]** well. Adjust the volume of all wells [S, IC, SC, EC and BC] to **80 µl/well** using BChE Assay Buffer. **Mix well and incubate at RT for 30 min, protected from light. Note:** Discard any unused, diluted BChE Enzyme after use.

3. Reaction Mix Preparation: Prepare a 12-fold dilution of the BChE Substrate (*i.e.* mix 5 µl of BChE Substrate with 55 µl of BChE Assay Buffer). Further dilute the diluted BChE Substrate at a 1:10 dilution. Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Reaction Mix containing:

	Reaction Mix
Diluted BChE Substrate	10 µl
Probe Mix	5 µl
BChE Assay Buffer	5 µl

Mix well and add 20 µl Reaction Mix to Sample **[S]**, Inhibitor Control **[IC]**, Enzyme Control **[EC],** Solvent Control **[SC]**, and Background Control **[BC]** wells and mix well. The total reaction volume in each well will be 100 µl.

4. Measurement: Measure the absorbance immediately in kinetic mode for 30-60 min at 412 nm at RT. Choose any two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding OD values for Sample (OD_1 and OD_2).

5. Calculation: Calculate the slope of Sample [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the $\Delta OD_{412\,nm}$ (OD_2 - OD_1) values over reaction time Δt (t_2-t_1). Subtract the slope of the Background Control [BC] values from the [S], [EC] and [SC] values. If the [SC] slope is significantly different from the [EC], use the [SC] value instead to calculate the effect of the test inhibitor(s).

$$\textbf{\% Relative Inhibition} = \frac{\textbf{Slope of}[\textbf{EC}] - \textbf{Slope of}\,[\textbf{S}]}{\textbf{Slope of}[\textbf{EC}]}\ \textbf{X 100}$$

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

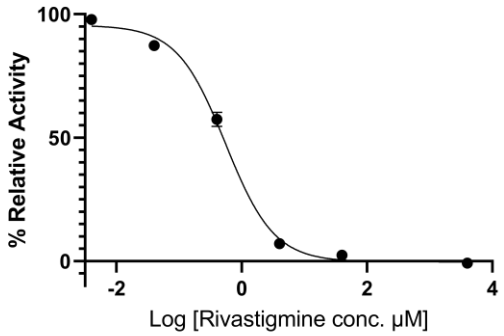


Figure: Inhibition of BChE activity by Rivastigmine. IC_{50} of Rivastigmine was calculated to be 550.9 ± 120.2 nM. Assay was performed following the kit protocol.

VII. Related Products:

Rivastigmine tartrate (B3093)
Acetylcholinesterase Inhibitor Screening Kit (Colorimetric) (K197)
Acetylcholinesterase Activity Colorimetric Assay Kit (K764)
Butyrylcholinesterase Activity Kit (K516)
Cholinesterase Activity Assay Kit (K975)
BCHE Antibody (Center) (6724)
Phosphate Buffered Saline (PBS) (2113)

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