ab283363 - Acetylcholinesterase Inhibitor Screening Kit (Colorimetric)

For the screening/characterizing of Acetylcholinesterase inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283363

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer 33	50 mL	-20°C
AChE Substrate	1 vial	-20°C
Acetylcholinesterase	1 vial	-20°C
DTNB	1 vial	-20°C
Donepezil	20 µl	-20°C

PLEASE NOTE: Assay Buffer 33 was previously labelled as Assay Buffer XXXIII and ACHE Assay Buffer, and DTNB as Probe Mix. The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening. Use within 1 year.

Assay Buffer 33: Warm to room temperature before use. Store at -20 °C.

AChE Substrate: Reconstitute in 100 µl of Assay Buffer 33. Store at -20 °C, protected from light.

Acetylcholinesterase: Reconstitute in 44 µl of Assay Buffer 33. Aliquot and store at -20 °C.

DTNB: Dissolve with 625 µl of Assay Buffer 33. Store at -20 °C.

Donepezil: Ready to use. Bring to room temperature before use.

Screening Protocol

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 Assay Buffer 33. 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 33 to designated well(s).

Inhibitor Control (Donepezil) [IC]:

1. 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 Assay Buffer 33, 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 Assay Buffer 33.
2. 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 Assay Buffer 33.

Item	[S]	[IC]	[EC]	[BC]
Test Inhibitor	10 µl	-	-	-
20 µM Donepezil	-	10 µl	-	-
Assay Buffer 33	-	-	100 µl	100 µl

Δ 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.*

AChE Enzyme Solution Preparation:

1. Prepare a 25-fold dilution of reconstituted Acetylcholinesterase (i.e. dilute 2 µl of Acetylcholinesterase with 48 µl of Assay Buffer 33), mix well.
2. 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 Assay Buffer 33 to well containing Background Control [BC].
3. Adjust the volume of each well to 160 µl/well with Assay Buffer 33. Mix well and incubate at room temperature for 10-15 min, protected from light.

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

Reaction Mix:

Prepare a 12-fold dilution of AChE Substrate (i.e. dilute of 4 µl of AChE Substrate with 44 µl of Assay Buffer 33). Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µl Reaction Mix containing:

Item	Reaction Mix
Diluted AChE Substrate	10 µl
DTNB	5 µl

Assay Buffer 33	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.

Measurement:
Measure absorbance (OD at 412 nm) immediately in kinetic mode for 40 minutes at room temperature. Choose two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).

Calculation:

1. Calculate the slope for all Sample Compounds [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the net ΔOD412 (OD2-OD1) values by the time Δt (t2-t1).
2. 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.

$$\%\ \boldsymbol{Inhibition} = \frac{Slope\ of\ EC\ -\ Slope\ of\ S}{Slope\ of\ EC} \times\ 100$$

$$\%\ \boldsymbol{Relative\ activity} = \frac{Slope\ of\ S}{Slope\ of\ EC} \times\ 100$$

Technical Support

For all technical or commercial enquiries please go to:
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