

Protocol of Acetylcholinesterase Enzyme-based Assay for High-throughput Screening

DOCUMENT: AChE_tox21_Version3.0
TITLE: Protocol of Acetylcholinesterase Enzyme-based Assay for High-throughput Screening

ASSAY REFERENCES:

Assay Target	Assay Readout	Assay Provider	Toxicity Pathway
Acetylcholinesterase	Absorbance	NCATS	neuron

QUALITY CONTROL PRECAUTIONS:

1. -The concentration of the substrate (acetylcholine) should be less than the K_m .
2. -The acetylcholinesterase used should also be optimized.
3. -The enzyme is plated into 1536-well plate first.
4. -Handle 1536 well plate black clear bottom plates carefully by sides

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-BiorapTR	-Beckman Coulter	-
-Envision	-Perkin Elmer	-
-Colorimetric reagents	-AAT Bioquest	- 11400
- Purified recombinant human AChE	- Sigma-Aldrich	- C168

PROCEDURE:

1 Assay Protocol

- 1.1 - Plate the enzyme in black-clear bottom 1536 well plate at 4uL for antagonist mode using a single tip plate dispenser (BiorapTR)
- 1.2 - Transfer 23nL of compounds from the library collection and positive control to the assay plates through pintool
- 1.3 -Incubate for 1 hr at room temperature
- 1.4 -Add 4 uL of cocktail solution (DTNB, acetylthiocholine) dye using a single tip plate dispenser (BiorapTR)
- 1.5 -Incubate at room temperature for 10 min in dark
- 1.6 -Read the absorbance intensity through Envision plate reader using protocol optimized