Technical Bulletin

Fluorimetric Acetylcholinesterase Assay Kit

Catalogue number MAK554

Product Description

Acetylcholinesterase, also known as AChE, is an enzyme that degrades (through its hydrolytic activity) the neurotransmitter acetylcholine, producing choline and an acetate group. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate synaptic transmission. AChE has a high catalytic activity- each molecule of AChE degrades about 5000 molecules of acetylcholine per second. Acetylcholinesterase is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen. Acetylcholinesterase exists in multiple molecular forms, which possess similar catalytic properties, but differ in their oligomeric assembly and mode of attachment to the cell surface.

The Fluorimetric Acetylcholinesterase Assay Kit provides a sensitive method for detecting AChE activity. The kit uses a Green Substrate to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE. The fluorescence intensity of the substrate is proportional to the formation of thiolcholine, thus the AChE activity.

Components

The kit is sufficient for 200 fluorometric assays in 96-well plates.

- Substrate, Green — 1 Vial
  Catalogue Number MAK554A
- Assay Buffer — 25 ml
  Catalogue Number MAK554B
- Acetylthiocholine — 1 Vial
  Catalogue Number MAK554C
- Acetylcholinesterase Standard — 1 Vial
  Catalogue Number MAK554D
- DMSO — 100 µL
  Catalogue Number MAK554E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader
- Black, flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Bovine Serum Albumin

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of Stock Solutions

Green Substrate Stock Solution (200X): Add 50 µL of DMSO into the vial of Substrate Green to make 200X Green substrate stock solution.

Acetylthiocholine Stock Solution (500X): Add 0.6 mL of purified water into the vial of Acetylthiocholine.

Acetylcholinesterase Standard Stock Solution (50 U/mL): Add 100 µL of purified water with 0.1% BSA into the vial of Acetylcholinesterase Standard to make a 50 U/mL Acetylcholinesterase Standard solution.

Preparation of Acetylcholinesterase Standards

1. Add 20 µL of the 50 U/mL Acetylcholinesterase Standard stock solution to 980 µL Assay Buffer to generate a 1000 mU/mL Standard solution.
2. Dilute the 1000 mU/mL Acetylcholinesterase Standard solution 1:10 using assay buffer to generate a 100 mU/mL Acetylcholinesterase Standard solution (AS1) per Table 1.
3. Perform 1:3 serial dilutions to obtain remaining diluted acetylcholinesterase Standards (AS2 – AS7) as per Table 1.

Note: Diluted acetylcholinesterase Standard solution is unstable and should be used within 4 hours.

Table 1.
Dilution of Acetylcholinesterase Standards

Dilution	AS Std Vol (µL)	Serial Dilution Source	Assay Buffer Vol (µL)	Conc (mU/mL)
AS1	22.5	from 1U/mL stock	202.5	100
AS2	75	From AS1	150	33.33
AS3	75	From AS2	150	11.11
AS4	75	From AS3	150	3.703
AS5	75	From AS4	150	1.234
AS6	75	From AS5	150	0.411
AS7	75	From AS6	150	0.137

Preparation of Acetylcholinesterase (AChE) Working Solution

Add 10 µL of Acetylthiocholine stock solution (500X) and 25 µL of Substrate, Green stock solution (200X) into 5 mL of Assay Buffer to make a total volume of 5.03 mL AChE Working Solution.

Note: The AChE working solution is unstable and should be used within 30 minutes. Protect from light.

Assay Reaction

1. Add 50 µL of each Acetylcholinesterase Standard, blank controls, and test Samples into a 96-well microplate. Use Assay Buffer for the blank control.

Note: For 384-well plates use 25 µL.

2. Add 50 µL of the AChE working solution to each well of acetylcholinesterase standard, blank control, and test Samples to make the total acetylcholinesterase assay volume of 100 µL/well.

Note: For 384-well plates use 25 µL, for a total assay volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

Measurement

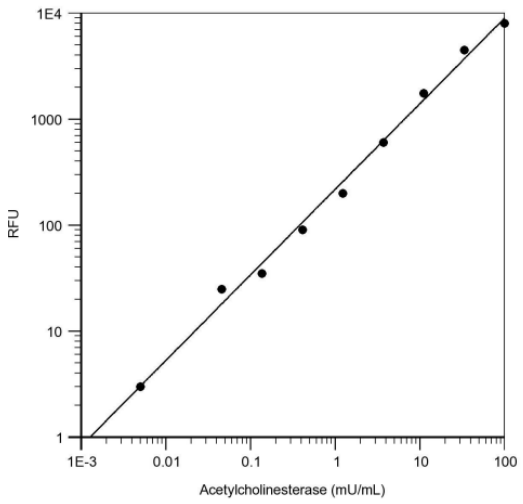
Monitor the fluorescence increase with a fluorescence microplate reader at $\lambda Ex/\lambda Em$ = 490/525 nm.

Results

1. The reading (RFU) obtained from the blank Standard well is used as a negative control.
2. Subtract the blank value from the Standards readings to obtain the base-line corrected values.
3. Plot the Standards readings to obtain a Standard curve and equation.
4. The concentration of Acetylcholinesterase in the test Samples may be determined from the Standard curve.

Figure 1.

Typical Acetylcholinesterase Standard Curve.



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Mak554pis Rev 12/23