ab138872

Acetylcholinesterase Assay Kit (Fluorometric - Green)

Instructions for Use

For the detection of Acetylcholinesterase activity in cell extracts, and in other solutions.

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Acetylcholinesterase (AChE) is one of the most crucial enzymes for nerve response and function. AChE degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate the synaptic transmission. AChE inhibitors are among the key drugs approved for Alzheimer's disease (AD) and myasthenia gravis.

ab138872 uses an outstanding Thiol Green Indicator to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE in cell extracts, and in other solutions. Thiol Green Indicator is not fluorescent until reacted with a thiol group. It has spectral properties similar to those of fluorescein, making this assay compatible with almost every fluorescence instrument. The fluorescence intensity of Thiol Green Indicator is used to measure AChE activity. Compared to the existing thiol probes (e.g., mBBr and bBBr), Thiol Green Indicator is much more sensitive.

ab138872 provides an ultrasensitive fluorometric one-step assay to detect as little as 0.01mU AChE in a 100 µL assay volume (0.1 mU/ml). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm. Our

Acetylcholinesterase Assay Kit (Fluorometric -Green) provides the most sensitive method for the detection of AChE activity.

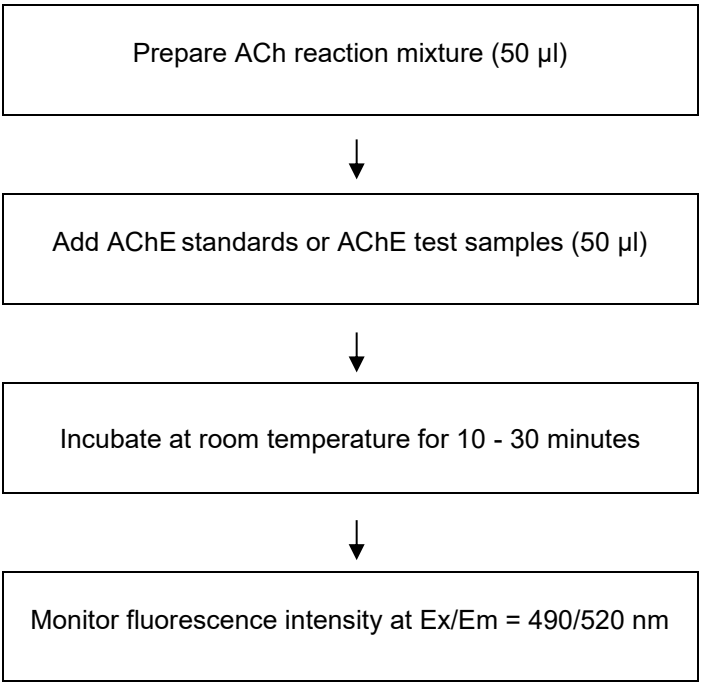
Kit Key Features

- **Broad Application:** Can be used to quantify acetylcholinesterase in solutions and in cell extracts.
- **Sensitive:** Detect as low as 0.01 mU of acetylcholinesterase in solution.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time.
- **Non-Radioactive:** No special requirements for waste treatment

This product does not differentiate between acetylcholinesterase (AchE) or butyrylcholinesterase (BChE) activity as both enzymes can hydrolyze acetylcholine.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: Thiol Green Indicator	1 vial
Component B: Assay Buffer	1 bottle (25ml)
Component C: Acetylthiocholine	1 vial
Component D: Acetylcholinesterase Standard	1 vial
Component E: DMSO	1 vial (100 μl)

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

- 96 or 384-well solid black microplates
- Fluorescence microplate reader
- ddH_2O
- 0.1% BSA
- Optional: AchE specific inhibitor. We recommend:
    - Territrem B (ab144370)
    - Donepezil hydrochloride (ab120763)
    - Cyclopenin (ab144233)

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Prepare Stock Solutions

1. 200X Thiol Green Indicator stock solution: Add 50 µl of DMSO (Component E) into the vial of Thiol Green Indicator (Component A) to make 200X Thiol Green Indicator stock solution.

   Note: The unused Thiol Green Indicator stock solution should be divided into single use aliquots. Store at -20 °C and avoid exposure to light

2. 500X Acetylthiocholine stock solution: Add 0.6 ml of ddH_2O into the vial of acetylthiocholine (Component C).

   Note: The unused acetylthiocholine stock solution should be divided into single use aliquots and stored at -20 °C.

3. Acetylcholinesterase standard stock solution: Add 100 µl of ddH_2O with 0.1% BSA into the vial of acetylcholinesterase standard (Component D) to make a 50 units/ml acetylcholinesterase stock solution.

Note: The unused acetylcholinesterase stock solution should be divided into single use aliquots and stored at -20 °C.

B. Prepare acetylthiocholine reaction mixture

Note: the acetylthiocholine reaction mixture is <u>not</u> stable and needs to be used within 30 min.

Prepare the acetylthiocholine reaction mixture according to Table 1 and keep from light.

Components	**Volume**
Assay Buffer (Component B)	5 ml
200X Thiol Green Indicator Stock Solution	25 µl
500X Acetylthiocholine Stock solution	10 µl
Total volume	5.03 ml

Table 1 Acetylthiocholine reaction mixture for one 96-well plate

C. Prepare serial dilutions of acetylcholinesterase standard (0 to 100 mU/ml):

1. Add 20 µl of 50 units/ml acetylcholinesterase standard stock solution to 980 µL of assay buffer (Component B) to generate 1000 mU/ml acetylcholinesterase standard solution.

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

2. Take 200 µl of 1000 mU/ml acetylcholinesterase standard solution to perform 1:10 and 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3, 0.1 and 0 mU/ml serially diluted acetylcholinesterase standards.

3. Add serially diluted acetylcholinesterase standards and/or acetylcholinesterase-containing test samples into a solid black 96-well microplate as described in Tables 2 and 3.

Note: Treat cells or tissue samples as desired.

BL	BL	TS	TS	….	….						
AS1	AS1	….	….	….	….						
AS2	AS2										
AS3	AS3										
AS4	AS4										
AS5	AS5										
AS6	AS6										
AS7	AS7										

Table 2. Layout of acetylcholinesterase standards and test samples in a solid black 96-well microplate.

Note: AS= Acetylcholinesterase *Standards; BL=Blank Control; TS=Test Samples*

Acetylcholinesterase Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µl	Assay Buffer: 50 µl	50 µl

Table 3. Reagent composition for each well.

**Note: Add the serial dilutions of acetylcholinesterase standard from 1 to100 mU/ml into wells from AS1 to AS7 in duplicate.*

D. Run acetylcholinesterase assay:

1. Add 50 µl of acetylthiocholine reaction mixture to each well of the acetylcholinesterase standard, blank control, and test samples to make the total acetylcholinesterase assay volume of 100 µl/well.

Note: For a 384-well plate, add 25 µl of sample and 25 µl of acetylthiocholine reaction mixture in each well.

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

3. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 490/520 nm.

NOTE: Butyrylcholinesterase (BChE) present in the sample can convert acetylcholine and lead to false positives. We recommend using a specific acetylcholinesterase as a control:

- Territrem B (ab144370)
- Donepezil hydrochloride (ab120763)
- Cyclopenin (ab144233)

7. Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and subtracted from the values for those wells with the acetylcholinesterase reactions. An acetylcholinesterase standard curve is shown in Figure 1.

Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

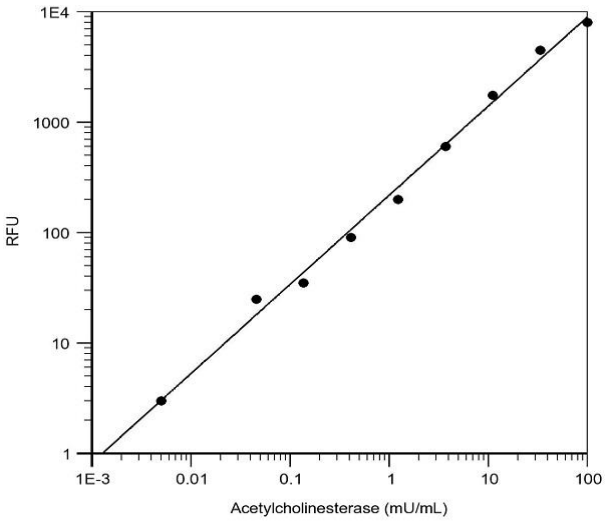


Figure 1. Acetylcholinesterase dose response was measured in a solid black 96-well plate with ab138872 using a fluorescence microplate reader. As low as 0.01 mU/well of acetylcholinesterase can be detected with 20 minutes incubation (n=3).

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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