ab138871

Acetylcholinesterase Assay Kit (Colorimetric)

Instructions for Use

For the detection of Acetylcholinesterase activity in red blood cell membranes, 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. Overview

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.

Abcam's Acetylcholinestarase Assay Kit (Colorimetric) (ab138871) provides a convenient method for the detection of AChE activity. It uses DTNB to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE in red blood cell membranes, in cell extracts, and in other solutions. The absorption intensity of DTNB adduct is used to measure the amount of thiocholine formed, which is proportional to the AChE activity. The kit provides a colorimetric one-step assay to detect as little as 0.1 mU AChE in a 100 µL assay volume (1 mU/ml). Its signal can be easily read by an absorbance microplate reader at ~410 nm. The kit is robust and can be used for continuously monitoring AChE activities.

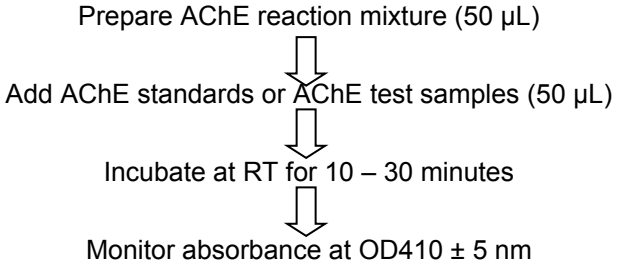
Kit Key Features

- **Broad Application:** Can be used to quantify acetylcholinesterase in solutions and in cell extracts.
- **Sensitive:** Detect as low as 0.1 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



3. Kit Contents

Components	**Amount**
Component A: DTNB	1 vial
Component B: Assay Buffer	1 bottle (25 mL)
Component C: Acetylthiocholine	1 vial
Component D: Acetylcholinesterase Standard	1 vial (5 units)

4. Storage and Stability

Upon arrival, store the kit at -20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

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

5. Materials Required, Not Supplied

- 96 – or 384-well white/clear microplates
- Microplate reader
- MilliQ or distilled water (ddH_2O)
- 0.1% BSA (Bovine Serum Albumin)
- (for serum samples): 3K-10K centrifugal filter
- (for plasma samples): heparin or citrate
- Triton X-100
- 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.

1. Reagent Preparation

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

a) 20X DTNB stock solution:

Add 0.6 mL of Assay Buffer (Component B) into the vial of DTNB (Component A) to make 20X DTNB stock solution.

Note: The unused DTNB stock solution should be divided into single use aliquots. Store at -20 °C and keep from light.

b) 20X Acetylthiocholine stock solution:

Add 0.6 mL of ddH2O into the vial of acetylthiocholine (Component C).

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

c) Acetylcholinesterase 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.

2. Prepare Samples

Treat cells or samples as desired according to experimental design prior collection.

a) Serum:

i. Collect blood, without using an anticoagulant. Allow blood to clot for 30 minutes at room temperature. Centrifuge at 2000x g at 4°C for 10 minutes.

ii. Remove the serum layer and store on ice. Take care to avoid disturbing the white buffy layer.

iii. Aliquot samples for testing and store remaining solution at -80°C.

iv. Prior to testing, filter samples with a 3K – 10K centrifugal filter.

v. Perform serum dilutions in Assay Buffer to ensure readings fall within the standard curve range.

b) Plasma:

i. Collect blood, using an anticoagulant (with heparin or citrate) and centrifuge at 1000x g. Allow blood to clot for 30 minutes at room temperature. Centrifuge at 2000x g at 4°C for 10 minutes.

ii. Remove the plasma layer and store on ice. Take care to avoid disturbing the white buffy layer.

iii. Aliquot samples for testing and store remaining solution at -80°C.

iv. Perform plasma dilutions in Assay Buffer. Plasma samples must be diluted at least 1:50 Assay Buffer for accurate determinations.

c) Plant cell lysates:

i. Homogenize the leaves with the lysis buffer at 200 mg/mL

ii. Centrifuge at 2500 rpm for 5-10 minutes and use the supernatant for the assay

d) Bacterial cell lysates:

i. Collect bacterial cells by centrifugation (10,000 x g, 0°C, 15 min)

ii. Use about 100 to 10 million cells/mL lysis buffer and leave at room temperature for 15 minutes.

iii. Centrifuge at 2500 rpm for 5 minutes and use the supernatant for the assay.

e) Mammalian cell lysates:

i. Remove medium from the plates (wells).

ii. Use about 100 uL lysis buffer per 1-5 million cells (or 100uL/ well in a 96-well cell culture plate), and leave at room temperature for 15 minutes.

iii. Use the cells directly, or centrifuge at 1500 rpm for 5 minutes and subsequently use the supernatant for the assay.

f) Tissue lysates:

i. Weigh around 20 mg tissue, wash with cold PBS, and homogenize with 400 µL of lysis buffer in a micro-centrifuge tube

ii. Centrifuge at 2500 rpm for 5-10 minutes and use the supernatant for the assay.

3. Prepare acetylthiocholine – reaction mixture

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

Components	**Volume**
Assay Buffer (Component B)	4.5 mL
20X DTNB Stock Solution	250 µL
20X Acetylthiocholine Stock solution	250 µL
Total volume	**5 mL**

Table 1. Acetylthiocholine reaction mixture for one 96-well plate

4. Prepare acetylcholinesterase standard (0 to 1000 mU/ mL):

a) Add 20 µL of 50 units/mL acetylcholinesterase stock solution (prepared in Section 6-1c) 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.

b) Use 1000 mU/ml acetylcholinesterase standard to perform dilutions of 300, 100, 30, 10, 3, 1 and 0 mU/ml serial dilutions of acetylcholinesterase standard.

c) Add serial dilutions of acetylcholinesterase standard and acetylcholinesterase-containing test samples into a white/clear bottom 96-well microplate as described in Tables 2 and 3.

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

Table 2. Layout of acetylcholinesterase standards (AS), test samples (TS) and blank control (BL) in a white/clear bottom 96-well microplate.

Acetylcholinesterase Standard	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 to1000 mU/ml into wells from AS1 to AS7 in duplicate.*

d) Add 5uL 10X (1uM) Donepezil hydrochloride to AChE sample well (45uL sample). Have a control well (5uL DMSO or solvent of your choice for 10X inhibitor + 45uL sample).

e) Incubate for 10 minutes

5. Run acetylcholinesterase assay:

a) 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.

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

c) Monitor the absorbance increase with an absorbance microplate reader at OD=410 ± 5 nm.

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

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

7. Data Analysis

a) Determine the average absorbance of each duplicate standard.

b) Subtract the absorbance value of the blank wells (with the assay buffer only) from itself and all other standards and samples. This is the corrected reading.

c) Plot the corrected reading values of each standard as a function of the amount of acetylcholinesterase. A typical acetylcholinesterase standard curve is shown in Figure 1.

d) Calculate the trendline equation based on your standard curve data.

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

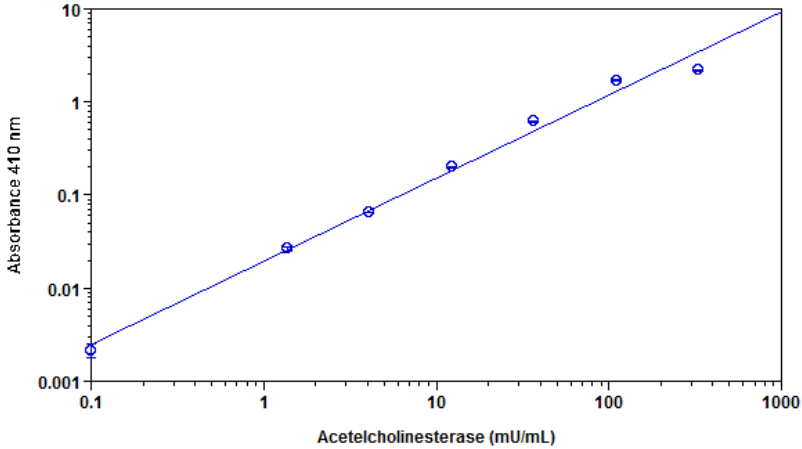


Figure 1. Acetylcholinesterase dose response was measured in a white/clear bottom 96-well plate with Acetylcholinesterase Assay Kit (Colorimetric) (ab138871) using a microplate reader. As low as 0.1 mU/well of acetylcholinesterase can be detected with 30 minutes incubation (n=3).

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 follow the deproteinization protocol
	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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