ab138871 Acetylcholinesterase Assay Kit (Colorimetric)

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.

For overview, typical data and additional information please visit: www.abcam.com/ab138871
(use abcam.cn/ab138871 for China, or abcam.co.jp/ab138871 for Japan)

Materials Supplied and Storage

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.

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)

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
- Lysis Buffer. We recommend:
  - Mammalian Cell Lysis Buffer 5X (ab179835)
- Optional: AchE specific inhibitor. We recommend:
  - Territrem B (ab144370)
  - Donepezil hydrochloride (ab120763)
  - Cyclopenin (ab144233)

1. Assay Procedure

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

1.1 Reagent Preparation

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

1.1.1 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.
If DTNB does not dissolve, heat to 37°C and vortex. If the precipitate forms/remains, increase the volume of buffer used to resuspend and adjust the amount of reagent used in the reaction mix. If precipitation persists, simply use the supernatant (without the undissolved particles). The use of the standard curve will allow for an accurate calculation of acetylcholinesterase activity.
Note: The unused DTNB stock solution should be divided into single use aliquots. Store at -20 °C and keep from light.

1.1.2 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.

1.1.3 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.

1.2 Prepare Samples

Treat cells or samples as desired according to experimental design prior collection. Please note that protease inhibitors may interfere with the assay.

1.2.1 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.

1.2.2 Plasma:

i. Collect blood using an anticoagulant such as heparin, or citrate.
ii. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80°C. The plasma sample will be stable for at least one month. Avoid repeated and freeze/thaw cycles.

1.2.3 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

1.2.4 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.

1.2.5 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.

1.2.6 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.

1.3 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

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

i. 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.

ii. 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.

iii. 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.*

iv. 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).

v. Incubate for 10 minutes

1.5 Run acetylcholinesterase assay:

i. 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.

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

iii. 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)

3. Data Analysis

i. Determine the average absorbance of each duplicate standard.

ii. 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.

iii. 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.

iv. 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.

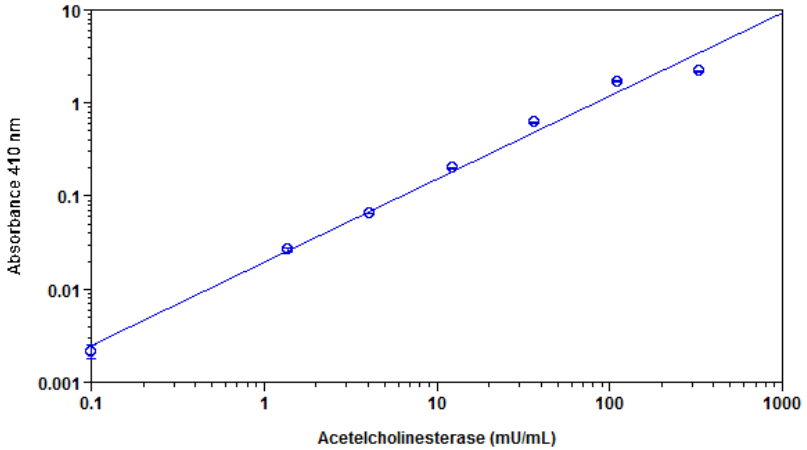

Figure 1. Acetylcholinesterase dose response was measured in a white/clear bottom 96-well plate with Acetylcholinesterase Assay Kit (Colorimetric) (ab138871) using a microplate reader. As low as 0.1 mU/well of acetylcholinesterase can be detected with 30 minutes incubation (n=3).

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)