

ab138871 Acetylcholinesterase Assay Kit (Colorimetric)

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.

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 (ddH₂O)
- 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)
 - Cyclophenin (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 ddH₂O 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.

Note: Whole blood samples are not recommended for use with this assay.

1.2.1 Serum:

- 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.
- Remove the serum layer and store on ice. Take care to avoid disturbing the white buffy layer.
- Aliquot samples for testing and store remaining solution at -80°C.
- Prior to testing, filter samples with a 3K – 10K centrifugal filter.
- Perform serum dilutions in Assay Buffer to ensure readings fall within the standard curve range.

1.2.2 Plasma:

- Collect blood using an anticoagulant such as heparin, or citrate.
- 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:

- Homogenize the leaves with the lysis buffer at 200 mg/mL
- Centrifuge at 2500 rpm for 5-10 minutes and use the supernatant for the assay

1.2.4 Bacterial cell lysates:

- Collect bacterial cells by centrifugation (10,000 x g, 0°C, 15 min)
- Use about 100 to 10 million cells/mL lysis buffer and leave at room temperature for 15 minutes.
- Centrifuge at 2500 rpm for 5 minutes and use the supernatant for the assay.

1.2.5 Mammalian cell lysates:

- Remove medium from the plates (wells).
- Use about 100 µL lysis buffer per 1-5 million cells (or 100µL/ well in a 96-well cell culture plate), and leave at room temperature for 15 minutes.
- 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:

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

