Elite™ Acetylcholinesterase Assay Kit (Green Fluorescence)

CATALOG NUMBER: CA-A401, 200 assays

Description

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.

This kit uses our outstanding Elite™ Green to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AChE in blood, in cell extracts, and in other solutions. Elite™ Green 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 Elite™ Green is used to measure AChE activity. Compared to the existing thiol probes (e.g., mBBr and bBBr), Elite™ Green is much more sensitive.

The Elite™ Acetylcholinesterase Assay Kit 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) as shown in Figure 1. 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 Elite™ Acetylcholinesterase Assay Kit provides the most sensitive method for the detection of AChE activity.

Kit Components

• **Component A:** Elite™ Green	1 vial
• **Component B:** Assay Buffer	1 bottle (25 ml)
• **Component C:** Acetylthiocholine	1 vial
• **Component D:** Acetylcholinesterase	1 vial (5 units)
• **Component E:** DMSO	1 vial (100 µl)

Storage

Keep **Component A** in freezer (-20 °C) and avoid exposure to light, **Component C&D** at -20 °C and **Component B&E** at 4 °C.

Materials Required (but not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescense microplate reader.

Assay Protocol

1. **Preparation of stock solution:**

   1.1 200X Elite™ Green stock solution: Add 50 µl of DMSO (**Component E**) into the vial of Elite™ Green (**Component A**) to make 200X Elite™ Green stock solution.

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

   1.2 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.

   1.3 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 standard stock solution.

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

2. **Preparation of acetylthiocholine reaction mixture:**

Prepare the acetylthiocholine reaction mixture according to the following table and keep from light.

Note: the acetylthiocholine reaction mixture is not stable, need be used within 30 min.

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

Components	**Volume**
Assay buffer (Component B)	5 ml
Elite™ Green stock solution (200x, from Step 1.1)	25 µl
Acetylthiocholine stock solution (500x, from Step 1.2)	10 µl
Total volume	**5.03 ml**

3. **Preparation of serially diluted acetylcholinesterase standards (0 to100 mU/ml)::**

3.1. Add 20 µl of 50 units/ml acetylcholinesterase standard stock solution (from Step 1.3) to 980 µl assay buffer (**Component C**) to generate 1000 mU/ml acetylcholinesterase standard solution.

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

3.2. 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.

3.3. Add serially diluted acetylcholinesterase standards and/or acetylcholinesterase-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Note: Treat cells or tissue samples as desired.

Table 1. Layout of Acetylcholinesterase standards and test samples in a solid black 96-well microplate

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

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

Table 2. Reagent composition for each well

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

Note: Add the serially diluted Acetylcholinesterase standards from 0.01 to 100 mU/ml into wells from AS1 to AS7 in duplicate.

4. Run acetylcholinesterase assay:

4.1. Add 50 µl of acetylthiocholine reaction mixture (from Step 2.1) into each well of the acetylcholinesterase standard, blank control, and test samples (see Step 3.3) 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 into each well.

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

4.3. Monitor the fluorescence increase at Ex/Em = 490/520 nm using a fluorescence plate reader.

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.

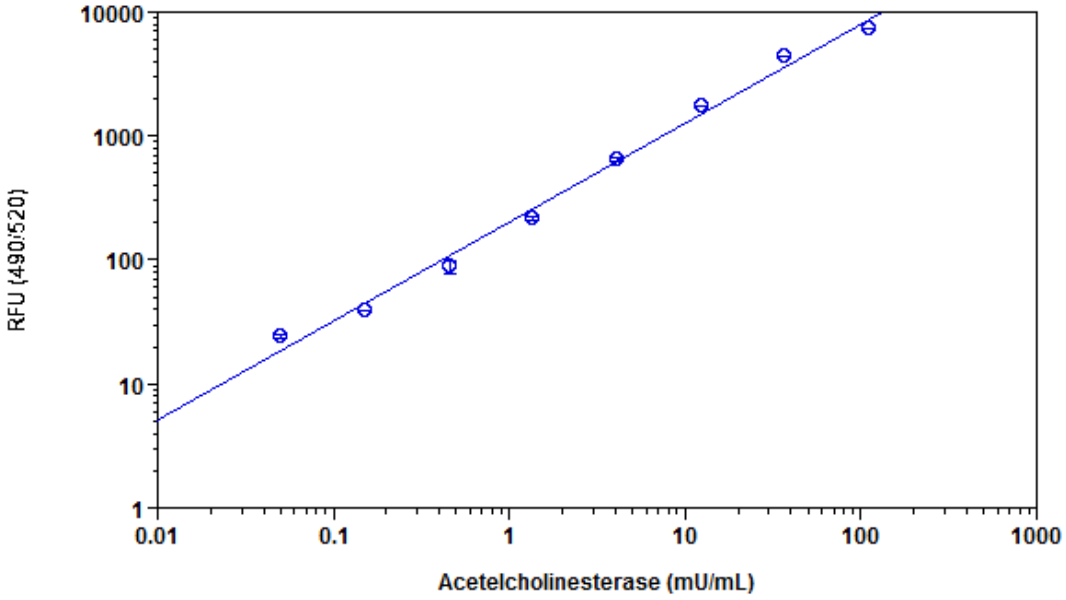

Figure 1. Acetylcholinesterase dose response was measured in a solid black 96-well plate with Elite™ Acetylcholinesterase Assay Kit using a Gemini fluorescence microplate reader (Molecular devices). As low as 0.01 mU/well of acetylcholinesterase can be detected with 20-minute incubation (n=3).

References

1. Kovarik, Z et al. (2003). Acetylcholinesterase active centre and gorge conformations analysed by combinatorial mutations and enantiomeric phosphonates. Biochem. J. (2003) 373, 33–40.
2. Ordentlich, A. et al. (1996). The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions withSelected Organophosphate Inhibitors. J. Biol. Chem. 271 (20):11953–11962.
3. Magnottl, RA. et al. (1987). Measurement of Acetylcholinesterase in Erythrocytes in the Field. Clin. Chem. 33/10, 1731-1 735.

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