Elite™ Acetylcholine Quantitation Kit (Red Fluorescence)

CATALOG NUMBER: CA-A403, 200 assays

Description

Acetylcholine (ACh) is a neurotransmitter substance with special excitatory properties of all preganglionic autonomic neurons, all parasympathetic postganglionic neurons and a few postganglionic sympathetic neurons. Acetylcholine is synthesized and liberated by the action of the enzyme choline acetyltranferase from the compounds choline and acetyl coenzyme A (acetyl CoA) which occurs in all cholinergic neurons. ACh exists only momentarily after its formation, being hydrolysed by the enzyme acetylcholinesterase which is present in the neurons of cholinergic nerves throughout their entire lengths and at neuromuscular junctions: this process is essential for proper muscle function as otherwise the accumulation of ACh would result in continuous stimulation of the muscles, glands and central nervous system. Alternatively, a shortage of ACh has devastating effect (e.g. myasthenia gravis).

The Elite™ Fluorimetric Acetylholine Quantiation Kit provides one of the most sensitive methods for quantifying acetylcholine. The kit uses Acetylcholine™ Red to quantify acetylcholine through the choline oxidase-mediated enzyme coupling reactions. The fluorescence intensity of Acetylcholine™ Red is proportional to acetylcholine formation. The kit is an optimized “mix and read” assay. It provides an ultrasensitive one-step fluorimetric assay to detect as little as 0.01 nanomoles ACh in a 100 µL assay volume (0.1 µM). Its signal can be easily read with a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~576 nm.

Kit Components

• **Component A:** Acetylcholine™ Red	1 vial
• **Component B:** Acetylcholine Probe	2 bottles (lyophilized powder)
• **Component C:** Acetylcholine Standard	1 vial
• **Component D:** Assay Buffer	1 bottle (25 ml)
• **Component E:** DMSO	1 vial (100 µl)

Storage

Keep in freezer (-20 ºC) and avoid exposure to light.

Materials Required (but not supplied)

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

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Assay Protocol

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

1. **Prepare stock solutions:**

   1.1 Acetylcholine™ Red stock solution (250X): Add 40 µl of DMSO (**Component E**) into the vial of Acetylcholine™ Red (**Component A**) to make a 250X stock solution.

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

   1.2 Acetylcholine stock solution: Add 200µl of ddH_2O into the vial of Acetylcholine Standard (**Component C**) to make 50 mM acetylcholine stock solution.

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

2. **Prepare acetylcholine assay mixture:**

   2.1 Add 5 ml of Assay Buffer (**Component D**) to the bottle of Acetylcholine Probe (**Component B**) and mix well.

   2.2 Add 20 µl of Acetylcholine™ Red stock solution (250X, from Step 1.1) into the Acetylcholine Probe bottle (from Step 2.1) to make the acetylcholine assay mixture.

   Note: *The Assay mixture should be used promptly and kept from light. The assay background would increase with longer storage time.*

3. **Prepare serial dilutions of acetylcholine standard (0 to100 µM):**

   3.1 Add 20 µl of 50 mM acetylcholine standard stock solution (from Step 1.2) to 980 µl Assay Buffer (Component D) to generate 1000 µM acetylcholine standard solution.

   Note: *Diluted acetylcholine standard solution is unstable, and should be used within 4 hours.*

   3.2 Take 200 µl of 1000 µM acetylcholine standard to perform 1:10 and 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3, 0.1 and 0 µM serial dilutions of acetylcholine standard.

   3.3 Add acetylcholine standards and acetylcholine containing test samples into a 96-well solid black microplate as described in Tables 1 and 2.

   Note: *Treat the cells or tissue samples as desired.*

Table 1. Layout of acetylcholine 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=Acetylcholine Standards; BL=Blank Control; TS=Test Samples.

Table 2. Reagent composition for each well

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

Note: Add the serially diluted Choline standards from 0.01 to 100 µM into wells from AS1 to AS7 in duplicate.

4. **Run the acetylcholine assay:**

   4.1. Add 50 µl of acetylcholine assay mixture (from Step 2.2) to each well of the acetylcholine standard, blank control, and test samples (see Step 3.3) to make the total acetylcholine assay volume of 100 µl/well.

   Note: *For a 384-well plate, add 25 µl sample and 25 µl of acetylcholine assay mixture per well.*

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

   4.3. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm.

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the acetylcholine reactions. An acetylcholine 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.

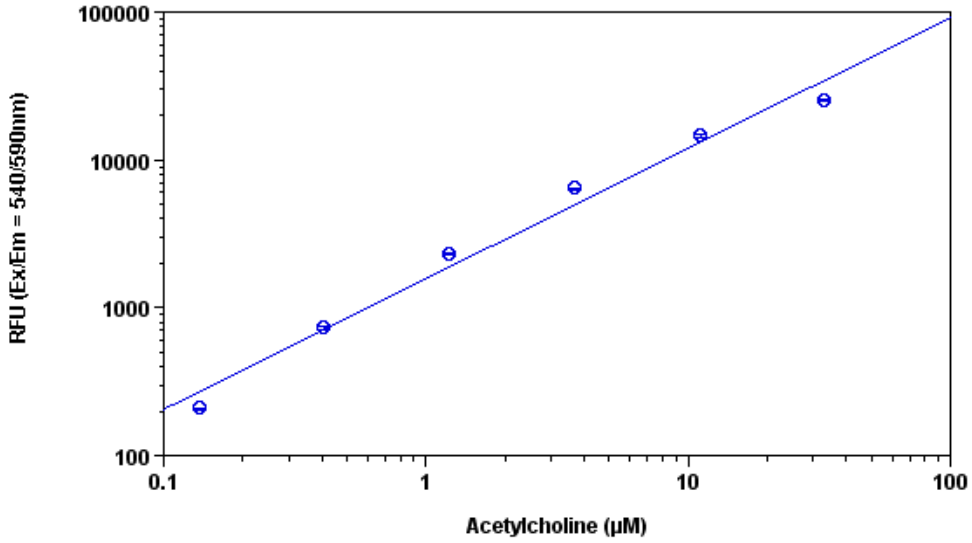

Figure 1. Acetylcholine dose response was obtained with Elite™ Acetylcholine Quantitation Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.1 µM (0.01 nmole/well) of acetylcholine can be detected with 10-minute incubation time (n=3).

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.