Amplite™ Fluorimetric Butyrylcholinesterase Activity Assay Kit

Catalog number: 11407
Unit size: 100 Tests

Component	Storage	Amount
Component A: Thiolite™ Green	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Refrigerate (2-8 °C)	1 bottle (25 mL)
Component C: Butyrylthiocholine	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: Butyrylcholinesterase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (0.25 U)
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 uL)

OVERVIEW

Butyrylcholinesterase (EC 3.1.1.8; BChE), also known as pseudocholinesterase or plasma cholinesterase, is mainly synthesized liver and present in blood. BChE is a nonspecific cholinesterase enzyme and can hydrolyze many different choline esters, serving as the first line of defense against toxic compounds reaching the bloodstream. It has been identified as a biomarker of organophosphate poisoning. Amplite™ Fluorimetric Butyrylcholinesterase Activity Assay Kit uses our outstanding Thiolite™ Green to quantify the generation of thiolcholine produced from the hydrolysis of butyrylthiocholine by BChE. The fluorescence intensity of Thiolite Green™ is proportional to the formation of thiolcholine, thus the BChE activity. The assay is convenient, sensitive and can detect as low as 0.1mU/mL in variety of samples.

AT A GLANCE

Protocol summary

1. Prepare BChE standards, dye working solution and test samples
2. Add BChE standards or test samples (100 uL)
3. Add BChE dye working solution (100 uL)
4. Incubate at room temperature for 10-30 minutes
5. Monitor fluorescence intensity at Ex/Em=490/525 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. *Thiolite™ Green stock solution (200X):*
   Add 50 uL of DMSO (Component E) into the vial of Thiolite™ Green (Component A) to make 200X Thiolite™ Green stock solution.

2. *Butyrylthiocholine (BTC) stock solution (100X):*
   Add 120 uL of ddH_2O into the vial of BTC (Component C) to make 100X BTC stock solution.

3. *Butyrylcholinesterase (BChE) Standard solution (5 U/mL):*
   Add 50 uL of ddH_2O with 0.1% BSA into the vial of BChE Standard (Component D) to make 5 U/mL BChE standard solution.

PREPARATION OF STANDARD SOLUTION

BChE standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11407

Add 20 uL of 5 U/mL BChE standard solution to 980 uL of Assay Buffer (Component B) to generate 100 mU/mL BChE standard solution (BS1). Then take 100 mU/mL BChE standard solution (BS1) and perform 1:3 serial dilutions in Assay Buffer(Component B) to get serially diluted BChE standards (BS2 - BS7). *Note:* Diluted BChE standard solution is unstable and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

BChE dye working solution:
Add 50 uL of 200X Thiolite™ Green stock solution and 100 uL of 100X BTC stock solution into 10 mL of Assay Buffer (Component B) to make a total volume of 10.1 mL BChE dye working solution.

Note Keep it from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of BChE standards and test samples in a clear bottom 96-well microplate. BS=BChE standards (BS1-BS7, 100 to 0.13 mU/mL); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
BS1	BS1	...	...
BS2	BS2	...	...
BS3	BS3		
BS4	BS4		
BS5	BS5		
BS6	BS6		
BS7	BS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
BS1-BS7	100 uL	Serial Dilutions (100 to 0.13 mU/mL)
BL	100 uL	Assay Buffer (Component B)
TS	100 uL	Test Sample

1. Prepare BChE standards (BS), blank controls (BL), and test samples (TS)

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according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 uL of reagent per well instead of 100 uL.

Note Treat cells or tissue samples as desired.

2. Add 100 uL of BChE dye working solution to each well of BChE standard, blank control, and test samples to make the total assay volume 200 uL/well. For a 384-well plate, add 25 uL of BChE working solution into each well instead, for a total volume of 50 uL/well.

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

4. Monitor the fluorescence increase with an fluorescence microplate reader at Ex/Em=490/525 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU (490/525nm)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate BChE samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

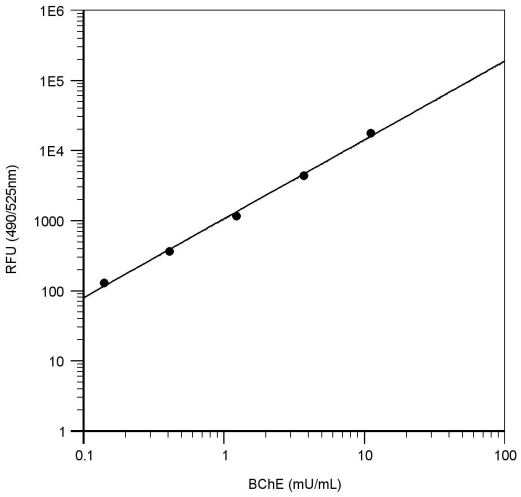

Figure 1. Butyrylcholinesterase dose response was measured in a solid black bottom 96-well plate with Amplite™ Fluorimetric Butyrylcholinesterase Assay Kit using a Gemini fluorescence microplate reader. As low as 0.1 mU/mL of Butyrylcholinesterase can be detected with 10 minutes incubation.

DISCLAIMER