Amplite™ Colorimetric Butyrylcholinesterase Activity Assay Kit

Catalog number: 11406
Unit size: 100 Tests

Component	Storage	Amount
Component A: DTNB	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 (1 U)

OVERVIEW

Butyrylcholinesterase (EC 3.1.1.8; BChE), also known as pseudocholinesterase or plasma cholinesterase, is mainly synthesized in 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 clinical biomarker of organophosphate poisoning. Amplite™ Colorimetric Butyrylcholinesterase Activity Assay Kit is based on a synthetic butyrylthiocholine-based substrate, which can be hydrolyzed by BChE and produce thiocholine. Thiocholine can react with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) and generates a yellow chromophore that can be detected at 410 nm. The assay is convenient, sensitive and can detect as low as 6 mU/mL in variety of samples.

AT A GLANCE

Protocol summary

1. Prepare BChE standards, test samples and dye working solution
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 absorbance at 410 ± 5 nm

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	410 ± 5 nm
Recommended plate:	Clear bottom

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. *DTNB stock solution (10X):*
   Add 1.2 mL of Assay Buffer (Component B) into the vial of DTNB (Component A) to make 10X DTNB stock solution. Keep from light.

   Note DNTB is not easy to dissolve, it is normal to see the cloudiness of the solution. One can use either the supernatant or the mixture for the experiment.

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 (20 U/mL):*
   Add 50 uL of ddH_2O with 0.1% BSA into the vial of Butyrylcholinesterase Standard (Component D) to make 20 U/mL butyrylcholinesterase Standard solution.

PREPARATION OF STANDARD SOLUTION

BChE standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11406

Add 20 uL of 20 U/mL BChE standard solution to 980 uL of Assay Buffer (Component B) to generate 400 mU/mL BChE standard solution (BS1). Then take 400 mU/mL BChE standard solution (BS7) and perform 1:2 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 1.0 mL of 10 X DTNB stock solutions and 100 µL of 100X BTC stock solution into 9 mL of Assay Buffer (Component B) to make a total volume of 10.1 mL BChE dye working solution. Keep away 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, 400 to 6.5 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 (400 to 6.5 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) 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.

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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 absorbance increase with an absorbance microplate reader at 410 ± 5 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance (410nm)) 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

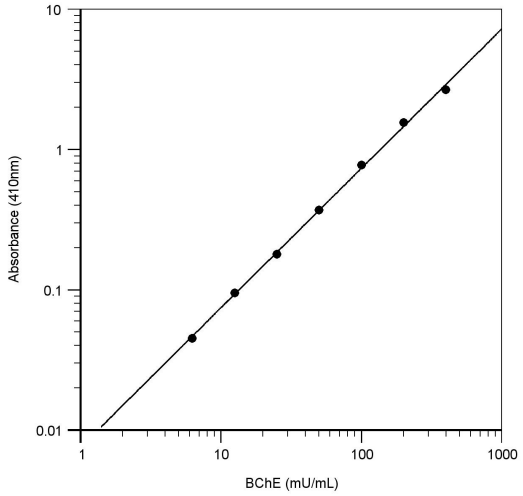

Figure 1. Butyrylcholinesterase dose response was measured in a white/clear bottom 96-well plate with Amplite™ Colorimetric Butyrylcholinesterase Assay Kit using a SpectraMax microplate reader. As low as 6.0 mU/mL of Butyrylcholinesterase can be detected with 10 minutes incubation (n=3).

DISCLAIMER