Technical Bulletin

Butyrylcholinesterase (BChE) Activity Assay Kit

Catalogue number MAK551

Product Description

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.

The 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.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- DTNB — 1 Vial
  Catalogue Number MAK551A
- Assay Buffer — 25 mL
  Catalogue Number MAK551B
- Butyrylthiocholine — 1 Vial
  Catalogue Number MAK551C
- Butyrylcholinesterase Standard — 1 Vial
  Catalogue Number MAK551D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- Bovine Serum Albumin (Catalogue number A7030 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of Stock Solutions

DTNB stock solution (10X): Add 1.2 mL of Assay Buffer into the vial of DNTB to make 10x DNTB Stock Solution keep from light.

Note: DNTB may appear cloudy after reconstitution and will not impact the assay reaction.

Butyrylthiocholine (BTC) stock solution (100X): Add 120 µL of purified water into the vial of Butyrylthiocholine to make 100X BTC stock solution.

Butyrylcholinesterase Standard Solution (20 U/mL): Add 50 µL of purified water with 0.1% BSA into the vial of Butyrylcholinesterase Standard to make a final concentration of 20 U/mL.

Preparation of Butyrylcholinesterase Standard Solution

1. Add 20 µL of 20 U/mL BChE standard solution to 980 µL of Assay Buffer to generate 400 mU/mL BChE standard solution (BS1).
2. Using the 400 mU/mL BChE standard solution (BS1), perform 1:2 serial dilutions in Assay Buffer to get serially diluted BChE standards (BS2 - BS7) as shown in Table 1.

Table 1:

Serial dilution of Butyrylcholinesterase Standard.

Dilution	BChE Std Vol (µL)	Assay Buffer Vol (µL)	Serial Dilution Source	Conc (mU/mL)
BS1	300	-	400 mU/mL stock	400
BS2	150	150	From BS1	200
BS3	150	150	From BS2	100
BS4	150	150	From BS3	50
BS5	150	150	From BS4	25
BS6	150	150	From BS5	12.5
BS7	150	150	From BS6	6.25

Note: Diluted Butyrylcholinesterase standard solutions are unstable and should be used within 4 hours.

Preparation of BChE Working Solution

1. Add 1.0 mL of 10 X DTNB stock solution and 100 µL of 100X BTC stock solution into 9 mL of Assay Buffer to make a total volume of 10.1 mL BChE Working Solution. Protect from light.

Assay Reaction

1. Add 100 µL of each standard, blank (Assay Buffer), and test sample to separate wells of a clear flat bottom 96 well plate. For a 384-well plate, use 25 µL.
2. Add 100 µL of BChE Working Solution to each well of BChE standard, blank, and test sample to make the total assay volume 200 µL/well. For a 384-well plate, use 25 µL of BChE working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction mixture at room temperature for 10 - 30 minutes protected from light.

Measurement

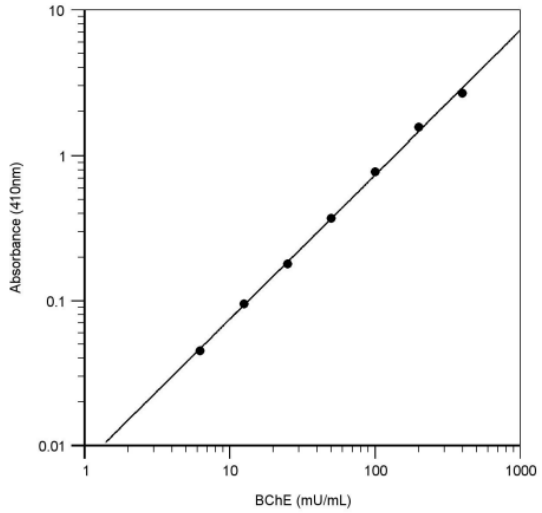
Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 410 nm ±5nm.

Results

1. The reading (Absorbance) obtained from the blank well is used as a negative control.
2. Subtract the blank value from the other standards' readings to obtain the base line corrected values.
3. Plot the standards readings to obtain the standard curve and equation.
4. The concentration of Butyrylcholinesterase present in the samples may be determined from the standard curve.

Figure 1.

Typical Butyrylcholinesterase Standard Curve



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