Technical Bulletin

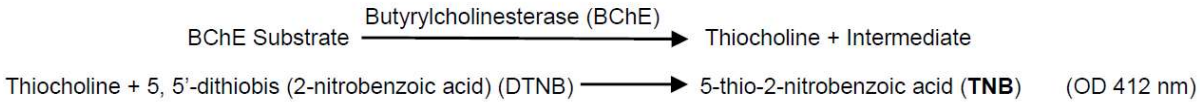
Butyrylcholinesterase Activity Kit (Colorimetric)

Catalog Number MAK406

Product Description

Butyrylcholinesterase (BChE), also known as plasma cholinesterase or pseudocholinesterase, is a serine hydrolase present in almost all mammalian tissues with the highest levels detected in plasma and liver. BChE hydrolyzes choline esters, as well as other esters and acts as an endogenous scavenger for anticholinesterase agents. BChE in plasma serves as the first line of defense against toxic compounds reaching the bloodstream that might inhibit acetylcholinesterase activity, which is essential in the nervous system. In clinical toxicology and clinical chemistry, determination of BChE activity in plasma is the most commonly used and preferred method to diagnose patients showing symptoms of intoxication. A study using genome-wide analysis suggested that BChE is a marker of obesity, insulin resistance and metabolic syndrome, hyperlipidemia, coronary artery disease, and hypertension. Serum BChE has been proposed as a marker of low-grade systemic inflammation and a marker of cardiovascular risk factor being even capable to predict mortality.

The Butyrylcholinesterase Activity Kit is based on the ability of BChE to hydrolyze an appropriate substrate and produce thiocholine. Thiocholine reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and generates a yellow chromophore that can be quantified at 412 nm. The assay is simple, sensitive and can detect as low as 0.2 U/mL in a variety of samples. The kit is suitable for the measurement of BChE activity and the screening of BChE inhibitors in biological samples. Biological samples include serum, plasma, blood and tissue homogenates (liver, lung, etc.).



Components

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

- BChE Assay Buffer 50 mL
  Catalog Number MAK406A
- BChE Substrate (in DMSO) 100 µL
  Catalog Number MAK406B
- Butyrylcholinesterase 1 vial
  Catalog Number MAK406C
- DTNB 1 vial
  Catalog Number MAK406D
- TNB Standard 1 vial
  Catalog Number MAK406E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
  96 well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Protease Inhibitor Cocktail (Catalog Number P8340)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening.

BChE Assay Buffer: Warm to room temperature prior to use. Store at 2-8 °C or -20 °C. If testing tissue samples, chill an appropriate amount of BChE Assay Buffer for use in Sample Preparation.

BChE Substrate: Aliquot and store at -20 °C, protected from light. Bring aliquot to room temperature prior to use.

Butyrylcholinesterase: Reconstitute vial in 20 µL of BChE Assay Buffer. Store at -20 °C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months of reconstitution.

DTNB Solution: Reconstitute DTNB with 625 µL of BChE Assay Buffer. Use within two months of reconstitution.

TNB Standard: Dissolve vial in 1 mL of BChE Assay Buffer to generate a 2.5 mM TNB Standard. The TNB standard solution is stable for at least two months at -20 °C.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum, plasma and blood

1. Prepare a 40-200-fold dilution of serum, plasma or blood in purified water, mix well.
2. Add 10-20 µL of diluted sample into desired well(s). Mix dilutions thoroughly by pipetting up and down after addition of biological fluids, since the density and viscosity cause sedimentation of sample to the bottom of the wells. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
3. Prepare identical parallel sample well(s) as Sample (S) for use as Sample Background Control (SBC).
4. Adjust the total volume of Sample and Sample Background Control wells to 95 µL with BChE Assay Buffer.

Tissue

1. Homogenize 10-30 mg of tissue with 100 µL of ice-cold BChE Assay Buffer containing 10 µL protease inhibitor cocktail (not included) and keep on ice for 10 minutes.
2. Centrifuge at 10,000 × *g* at 4 °C for 10 minutes to remove cell debris.
3. Transfer the supernatant to a fresh tube.
4. Add 5-20 µL of sample per well. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.

5. Prepare identical parallel sample well(s) as Sample (S) for use as Sample Background Control (SBC).
6. Adjust the total volume of Sample and Sample Background Control wells to 95 µL with BChE Assay Buffer.

BChE Positive Control

1. Prepare a 50-fold dilution of Butyryl-cholinesterase solution (e.g., dilute 1 µL of Butyrylcholinesterase stock solution with 49 µL of BChE Assay Buffer).
2. Add 8-12 µL of Diluted Butyryl-cholinesterase from Step 1 into well(s) assigned as BChE Positive Control.
3. Adjust the total volume of the Positive Control well(s) to 95 µL with BChE Assay Buffer.

Standard Curve Preparation

Prepare TNB Standards in duplicate according to Table 1.

Table 1.
Preparation of TNB Standards

Well	2.5 mM TNB Standard	BChE Assay Buffer	TNB (nmol/well)
1	0 µL	200 µL	0
2	2 µL	198 µL	5
3	4 µL	196 µL	10
4	6 µL	194 µL	15
5	8 µL	192 µL	20
6	10 µL	190 µL	25
7	12 µL	188 µL	30

DTNB

1. Add 5 µL of DTNB Solution to each well containing Sample (S), Sample Background Control (SBC), and Positive Control. The total volume in each well (Sample, Sample Background Control and Positive Control) at this step should be 100 µL. Note DTNB solution is **not** added to Standard wells.
2. Incubate the plate for 10 minutes at room temperature, protected from light, to achieve temperature equilibrium and complete the reaction of sample proteins' sulfhydryl groups with DTNB.

BChE Substrate Preparation

1. Prepare a 120-fold dilution of BChE substrate by diluting 5 µL of BChE Substrate with 595 µL of BChE Assay Buffer.
2. Vortex briefly and keep on ice.
3. Add 100 µL of Diluted BChE Substrate from Step 2 to each well containing the Sample (S) and BChE Positive Control. Mix well.
4. For Sample Background Control (SBC), add 100 µL of BChE Assay Buffer into assigned well(s). Do not add Diluted BChE Substrate to SBC wells.
5. The total volume in every well (standards, Samples, and Positive Controls and Sample background Controls) should now be 200 µL.
6. Carefully shake the plate for 10 seconds to mix contents prior to start of read-out.

Measurement

Immediately measure absorbance at 412 nm (A_{412}) in kinetic mode for 20-30 minutes at room temperature. The TNB Standard Curve can be read in endpoint mode.

Results

1. Subtract the 0 Standard reading from **all** readings, including Sample (S) and Sample Background Control (SBC).
2. Plot the TNB Standard Curve.
3. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the absorbance (A_1 and A_2).
4. Calculate ΔA (ΔA= A_2-A_1).
5. Apply the ΔA for all Samples and paired Sample Background Controls to the TNB Standard Curve to get B nmol of TNB generated during the reaction time ΔT.

6. Calculate ΔT ($\Delta T = T_2 - T_1$).
7. Subtract the amount of TNB generated by the Sample Background Control (SBC) from the amount of TNB generated by its paired Sample (S) over the same time period to calculate the Sample BChE activity:

Sample BChE Activity (nmol/min/mL or mU/mL) =

$$\frac{(B_{Sample} - B_{SBC})}{\Delta T \times V} \times D$$

where:

B = TNB amount from Standard Curve (nmol)

ΔT = Reaction time (minutes)

V = Sample volume added into the reaction well (mL)

D = Dilution Factor

Unit Definition: One unit of BChE activity is the amount of enzyme that generates 1.0 μmol of Thiocholine per minute at pH 7.4 at room temperature.

Figure 1.
Typical TNB Standard Curve

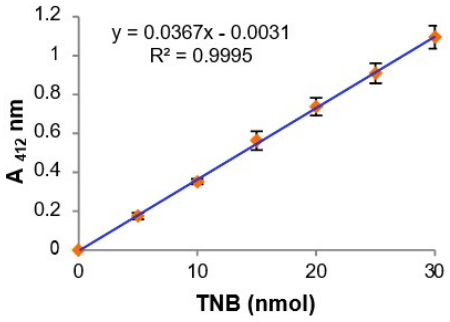


Figure 2.
BChE activity in Human Blood (10 μL, 1:100 dilution), Human Serum (10 μL, 1:50 dilution) and Human Plasma (10 μL, 1:50 dilution)

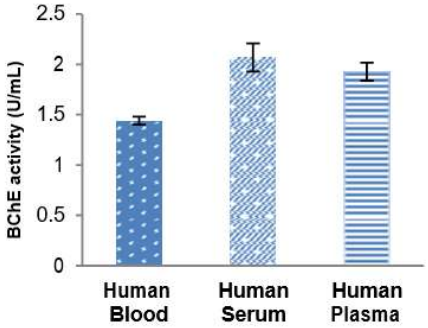
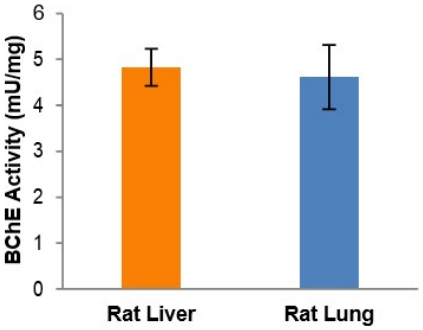


Figure 3.
BChE activity in Rat Liver (30 μg protein) and Rat Lung (15 μg protein). Assays were performed following the kit protocol.



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