Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Butyrylcholinesterase Activity Kit (Colorimetric)

8/18

(Catalog # K516-100; 100 assays; Store at -20°C)

I. Introduction:

Butyrylcholinesterase (EC 3.1.1.8; 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 recent 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. Biovision's Butyrylcholinesterase Activity Kit is based on the ability of BChE to hydrolyze 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 variety of samples.

BChE Substrate $\xrightarrow{\text{Butyrylcholinesterase (BChE)}}$ Thiocholine + Intermediate

Thiocholine + 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) $\longrightarrow$ 5-thio-2-nitrobenzoic acid (**TNB**) (OD 412 nm)

II. Applications:

- Measurement of BChE activity in biological samples
- Screening of BChE inhibitors by using biological samples

III. Sample Type:

- Serum, plasma or blood
- Tissue homogenates: liver, lung, etc.

IV. Kit Contents:

Components	K516-100	Cap Code	Part Number
BChE Assay Buffer	50 ml	NM	K516-100-1
BChE Substrate (in DMSO)	100 µl	Blue	K516-100-2
Butyrylcholinesterase	1 vial	Green	K516-100-3
DTNB	1 vial	Red	K516-100-4
TNB Standard	1 vial	Brown	K516-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store Kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **BChE Assay Buffer:** Warm to room temperature before use. Store at 4 ºC or -20 ºC.
- **BChE Substrate:** Aliquot and store at -20 °C, protect from light. Bring to room temperature before use.
- **Butyrylcholinesterase:** Reconstitute Butyrylcholinesterase in 20 µl BChE Assay Buffer. Store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within 2 months.
- **DTNB Solution:** Dissolve DTNB with 625 µl Assay Buffer. Use within 2 months.
- **TNB Standard:** Dissolve in 1 ml of Assay Buffer to generate 2.5 mM TNB Standard. The TNB standard solution is stable for at least 2 months at -20°C.

VII. Butyrylcholinesterase Activity Assay Protocol:

1. Sample Preparation:

a. For serum, plasma or blood: Prepare a 40-200 fold dilution of serum, plasma or blood in dH_2O. Add 10-20 µl of diluted sample into desired well(s). Prepare parallel sample well(s) as Sample Background Control (See step 4).

b. For tissues: Homogenize tissue (10-30 mg) with 100 µl ice-cold BChE Assay Buffer containing protease inhibitor cocktail (Cat. # K272 or equivalent), and keep on ice for 10 min. Centrifuge at 10,000 x g at 4 ºC for 10 min to remove cell debris. Transfer the supernatant to a fresh tube. Add 5-20 µl sample per well. Prepare parallel sample well(s) as Sample Background Control (See step 4).

c. For BChE positive control: Prepare a 50-fold dilution of Butyrylcholinesterase solution (i.e. Dilute 1 µl of Butyrylcholinesterase stock solution with 49 µl BChE Assay Buffer). Add 8-12 µl of Diluted Butyrylcholinesterase into well(s) assigned as BChE Positive Control.

Adjust the volume of sample(s), background control(s) and Positive Control to 95 µl/well BChE Assay Buffer.

Notes:

a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve Range.

b. 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.

2. **TNB Standard Curve:** Add 0, 2, 4, 6, 8, 10, 12 µl of the 2.5 mM TNB Standard into 96-well plate in duplicate to generate 0, 5, 10, 15, 20, 25, 30 nmol/well standard. Bring the final volume to 200 µl with BChE Assay Buffer.
3. **DTNB:** Add 5 µl of DTNB solution to each well containing the test samples, Sample Background Control, BChE Positive Control. *The total volume in every well (i.e. samples, background controls) should be 100 µl*. **Incubate plate for 10 min at room temperature** to achieve temperature equilibrium and complete the reaction of sample proteins' sulfhydryl groups with DTNB, avoid light.
4. **BChE substrate preparation:** Prepare a 120-fold dilution of BChE substrate (i.e. Dilute 5 µl of BChE stock substrate with 595 µl BChE Assay Buffer), vortex briefly and keep on ice. Add 100 µl of Diluted BChE substrate to each well containing the test samples and BChE Positive Control. Mix well. For Sample Background Control, add 100 µl BChE Assay Buffer into assigned well(s). The total volume in every well (i.e. standards, samples, background controls) should be 200 µl.
5. **Measurement:** Measure absorbance immediately at 412 nm in kinetic mode for 20-30 min at room temperature. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD1 and OD2). The TNB Standard Curve (step 2) can be read in Endpoint mode.

   Note: Shake the microplate carefully for 10 seconds to mix contents prior to start of read-out.
6. **Calculation:** Subtract 0 Standard reading from all readings. Plot the TNB Standard Curve. Calculate the BChE activity of the test sample: Δ**OD = OD$_2$ – OD$_1$**. Apply the ΔOD to the TNB Standard Curve to get B nmol of TNB generated during the reaction time (Δ**t = t$_2$ - t$_1$**). Subtract the sample background control reading from its paired sample reading (**B $_{\text{test sample}}$/Δt-B $_{\text{sample background control}}$/$\Delta$t**).

$$\textbf{Sample BChE Activity} = \frac{(\text{B test sample} - \text{B sample control})}{\Delta t \cdot V} * \text{D} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** = TNB amount from Standard Curve (nmol)
Δ**t** = Reaction time (min.)
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 min. at pH 7.4 at room temperature.

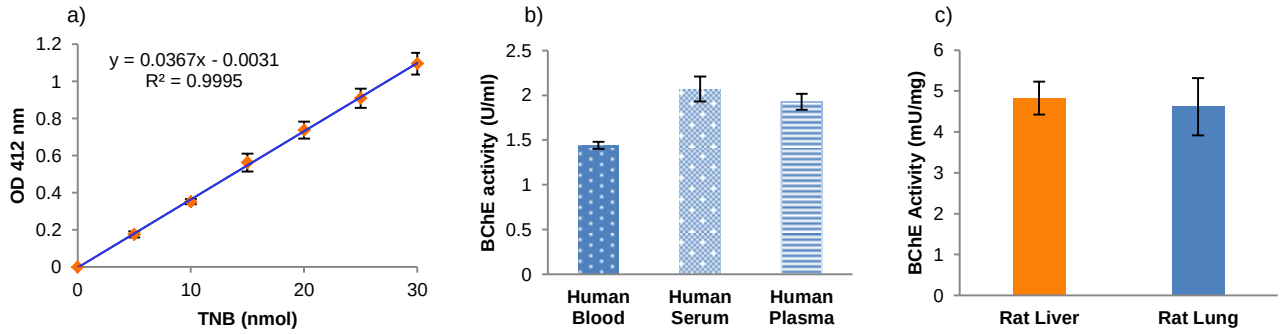

Figure: (a) TNB standard curve; (b) BChE activity in Human Blood (10 µl, 1:100 dilution), Human Serum (10 µl, 1:50 dilution) and Human Plasma (10 µl, 1:50 dilution); (c) BChE activity in Rat Liver (30 µg protein) and Rat Lung (15 µg protein). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Acetylcholinesterase Activity Colorimetric Assay Kit (K764-100)
Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615-100)

FOR RESEARCH USE ONLY! Not to be used on humans.