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Cholinesterase Activity Assay Kit (Colorimetric)

rev 06/21

(Catalog # K975-100; 100 assays; Store at -20 °C)

I. Introduction:

Cholinesterase (ChE) consists of a group of enzymes that hydrolyze choline esters. There are two ChE isoenzymes in blood: acetylcholinesterase (AChE; EC 3.1.1.7), also known as erythrocytes or true ChE, which is found mainly in red blood cells; and butyrylcholinesterase (BChE; EC 3.1.1.8), also known as plasma ChE or pseudo-ChE, which is present in plasma. Blood AChE or BChE activity would be selectively reduced by exposing them to poisonous chemical agents, insecticides such as organophosphates or carbamates, anesthetics, and a variety of therapeutic drugs including donepezil or rivastigmine which are used for treating Alzheimer's diseases. Therefore, Blood Cholinesterases (ChE=AChE+BChE) are potential biomarkers of suppressed and/or increased central and peripheral nervous system activity and tools for confirming possible therapeutics. Since plasma BChE and erythrocyte AChE can be selectively inhibited by certain insecticides or drugs, quantification of both isoenzymes' activities is important. **BioVision's cholinesterase activity kit** combines the specific AChE and BChE substrates and a selective BChE inhibitor to measure and distinguish AChE and BChE activities in Whole Blood samples without separating plasma from erythrocytes. The principle is based on the ability of AChE and BChE to hydrolyze their respective substrates and produce thiocholine. Thiocholine reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) generating a yellow chromophore (TNB) that can be quantified at 412 nm. It is simple, easy to implement, and useful in clinical research to monitor exposure to anti-ChE compounds in Blood Samples.

AChE Activity in Sample: AChE substrate —(BChE Inhibitor)→ Thiocholine + Intermediate
Thiocholine + (DTNB) → **TNB** (OD 412 nm)

BChE Activity in Sample: BChE substrate → Thiocholine + Intermediate
Thiocholine + (DTNB) → **TNB** (OD 412 nm)

II. Applications:

- Measurement of ChE activity in Biological Fluids
- Screening of ChE inhibitors in Biological Samples

III. Sample Type:

- Biological Fluids: Blood

IV. Kit Contents:

Components	K975-100	Cap Code	Part Number
ChE Assay Buffer	100 ml	NM	K975-100-1
AChE Substrate	1 vial	Purple	K975-100-2
BChE Substrate (in DMSO)	100 µl	Blue	K975-100-3
Acetylcholinesterase	1 vial	Orange	K975-100-4
Butyrylcholinesterase	1 vial	Green	K975-100-5
BChE Inhibitor	1 vial	White	K975-100-6
DTNB	2 vials	Red	K975-100-7
TNB Standard	1 vial	Amber	K975-100-8

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

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

- **ChE Assay Buffer:** Store at 4 ºC or -20 ºC. Bring to room temperature (RT) before use.
- **AChE Substrate:** Reconstitute the vial in 100 µl ChE Assay Buffer. Store at -20 ºC. Use within two months.
- **BChE Substrate:** Store at -20 °C, protected from light. Bring to RT before use.
- **Acetylcholinesterase:** Reconstitute the vial in 100 µl of ChE Assay Buffer. Divide into aliquots and store at -20 ºC. Use within two months.
- **Butyrylcholinesterase:** Reconstitute the vial in 20 µl ChE Assay Buffer. Store at -20 ºC. Use within two months.
- **BChE Inhibitor:** Reconstitute the vial in 150 µl **dH_2O**. Vortex intensively at RT to facilitate solubilization. Divide into aliquots and store at -20ºC. Bring to RT before use. Use it within two months.
- **DTNB Solution:** Dissolve 1 vial of DTNB in 625 µl ChE Assay Buffer. Each vial can be used to carry out up to 50 reactions. Dissolve the vial contents when needed. Store at -20 ºC. Use within two months.
- **TNB Standard:** Dissolve the vial in 1 ml of ChE Assay Buffer to generate 2.5 mM TNB Standard. Use within two months.

VII. Cholinesterase Assay Protocol:

1. BChE inhibitor, Sample Preparations:

a. BChE inhibitor**:** Dilute BChE Inhibitor 15-fold (i.e. Dilute 10 µl BChE Inhibitor with 140 µl ChE Assay Buffer).

b. Blood sample: Prepare a 40-200 fold dilution of Blood in **dH_2O**. *Record Dilution Factor.* Add 10-20 µl of Diluted Blood into 3

parallel well(s) assigned as **Sample$_{AChE}$**, **Sample$_{BChE}$** and **Sample$_{control}$**, respectively. Add 20 µl of Diluted BChE Inhibitor into the sample well assigned as **Sample$_{AChE}$**. Add 20 µl of ChE Assay Buffer into the other 2 sample well(s) assigned as **Sample$_{BChE}$** and **Sample$_{control}$**. *These experimental conditions will lead to direct estimation of AChE Activity.*

c. For **AChE Positive Control:** Dilute the reconstituted Acetylcholinesterase solution 50-fold in ChE Assay Buffer. Add 8-12 µl of diluted Acetylcholinesterase into the desired well(s) assigned as **AChE Positive Control**.

d. For **BChE Positive Control** and **BChE Inhibitor Positive Control:** Dilute the reconstituted Butyrylcholinesterase solution 50-fold in ChE Assay Buffer. Add 8-12 µl of diluted Butyrylcholinesterase into 2 separate wells. Add 20 µl of diluted BChE Inhibitor into one well, assigned as **BChE Inhibitor Positive Control** and add 20 µl of ChE assay buffer into the other well assigned as **BChE Positive Control**. Adjust the volume of **Sample$_{AChE}$**, **Sample$_{BChE}$**, **Sample$_{control}$**, **AChE Positive Control, BChE Positive Control, BChE Inhibitor Positive Control** to 95 µl/well with ChE Assay Buffer.

Notes:

a. It is important to mix dilutions thoroughly by pipetting up and down after addition of Blood samples, since the density and viscosity cause sedimentation of sample to the bottom of the wells.

b. Screening of ChE inhibitors in Blood: High solvent concentration might affect the AChE or BChE enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity (such as in the presence of final solvent concentration).

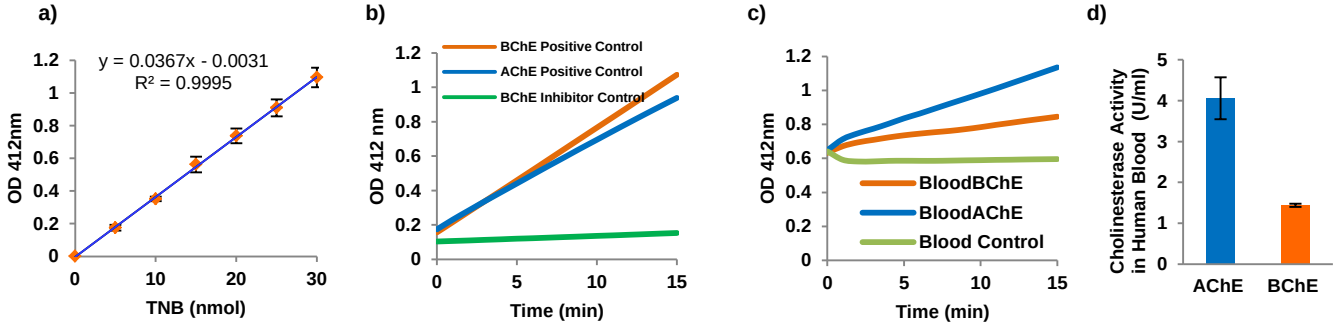
2. **TNB Standard Curve:** Add 0, 2, 4, 6, 8, 10, 12 µl of the TNB Standard into a 96-well plate in duplicates to generate 0, 5, 10, 15, 20, 25, 30 nmol/well standard. Adjust the final volume to 200 µl with ChE Assay Buffer.
3. **DTNB:** Add 5 µl DTNB solution to each well containing **Sample$_{AChE}$**, **Sample$_{BChE}$**, **Sample$_{control}$**, **AChE Positive Control, BChE Positive Control, BChE Inhibitor Positive Control**. *The total volume in every well should be 100 µl*. **Incubate the plate for 10 min at RT with gentle shaking**, protected from light.
4. **AChE and BChE Substrate preparation:** Prepare a 120-fold dilution of reconstituted AChE Substrate and a 120-fold dilution of BChE Substrate, respectively (i.e. add 5 µl of each of the substrates with 595 µl ChE Assay Buffer) and vortex briefly. Add 100 µl of diluted AChE Substrate to wells containing **Sample$_{AChE}$**, **AchE Positive Control**. Add 100 µl of diluted BChE Substrate to wells containing **Sample$_{BChE}$**, **BChE Positive Control** and **BChE Inhibitor Positive Control**. Add 100 µl of ChE Assay Buffer to **Sample$_{control}$** well. Mix well.
5. **Measurement:** Measure the absorbance immediately at 412 nm in kinetic mode for 10-30 min at RT. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding absorbance values (OD_1 and OD_2). The TNB Standard Curve (see step 2) can be read in Endpoint mode.

   Note: We suggest carefully shake the microplate for 10 sec to mix the contents prior to the start of the measurement.
6. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the TNB Standard Curve. Calculate the AChE and BChE activity of the test sample: $\Delta OD = OD_2 - OD_1$. Apply the ΔOD to the TNB Standard Curve to get B nmol of TNB generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\textbf{Sample AChE/BChE Activity} = \frac{(\textbf{B Sample (AChE or BChE)} - \textbf{B Sample (Control)})}{\Delta \mathbf{t} \cdot \mathbf{M}} * D = \textbf{nmol/min/ml = mU/ml}$$

Where: **B** = TNB amount from Standard Curve (nmol)
Δ**t** = Reaction time (min.)
M = Sample total volume added into the reaction well (ml)
D = Dilution Factor

Unit Definition: One unit of AChE/BChE activity is the amount of enzyme that generates 1.0 nmol of Thiocholine per min. at pH 7.5 at RT.



FigureS: (a) TNB Standard Curve. (b) Measurement of purified BChE activity with or without BChE inhibitor and purified AChE activity. (c) & (d) AChE and BChE activity in human blood (10 µl, 1:50 dilution). Assays were performed following the kit protocol.

VIII. Related Products:

Acetylcholinesterase Activity Colorimetric Assay Kit (K764-100)
Butyrylcholinesterase Activity Kit (K516-100)
Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615-100)

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