Version 2a, Last updated 22 August 2023

ab235937 Cholinesterase Activity Assay Kit (Colorimetric)

For the measurement of cholinesterase in biological fluids such as blood.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Cholinesterase Activity Assay Kit (Colorimetric) (ab235937) combines the specific acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) 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- Cholinesterase compounds in blood Samples.

Prepare samples, BChE inhibitor, AChE Positive Control, BChE Positive Control and BChE Inhibitor Positive Control.

⇩

Prepare TNB Standard Curve.

⇩

Add DTNB solution to each well containing $Blood_{AChE}$, $Blood_{BChE}$, $Blood_{control}$, AChE, BChE and BChE Inhibitor Positive Control.

⇩

Prepare AChE and BChE substrates.

⇩

Measure absorbance immediately at 412 nm in kinetic mode for 10-30 min at room temperature.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer XLI/ChE Assay Buffer	100 mL	-20°C	4°C or -20°C
AChE Substrate	1 vial	-20°C	-20°C
BChE Substrate/BChE Substrate (in DMSO)	100 µL	-20°C	-20°C
AChE Positive Control/Acetylcholinesterase	1 vial	-20°C	-20°C
BChE Positive Control/Butyrylcholinesterase	1 vial	-20°C	-20°C
BChE Inhibitor	1 vial	-20°C	-20°C
DTNB	2 vials	-20°C	-20°C
TNB Standard	1 vial	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Assay Buffer XLI/ChE Assay Buffer

1. Ready to use as supplied.
2. Warm to room temperature before use.

5.2 AChE Substrate

1. Reconstitute in 100 µL Assay Buffer XLI/ChE Assay Buffer.
2. Store at -20°C. Use within two months.

5.3 BChE Substrate/BChE Substrate (in DMSO)

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at -20°C in dark.

5.4 AChE Positive Control/Acetylcholinesterase

1. Reconstitute with 100 µL of Assay Buffer XLI/ChE Assay Buffer.
2. Aliquot and store at -20 °C. Use within two months.

5.5 BChE Positive Control/Butyrylcholinesterase

1. Reconstitute with 20 µL Assay Buffer XLI/ChE Assay Buffer.
2. Store at -20 °C. Use within two months.

5.6 BChE Inhibitor

1. Reconstitute BChE Inhibitor in 150 µL dH_2O.
2. Vortex intensively at room temperature to facilitate solubilization.
3. Aliquot and store at -20°C. Use it within two months.
4. Bring to room temperature before use.

5.7 DTNB Solution

1. Dissolve 1 vial of DTNB with 625 µL Assay Buffer XLI/ChE Assay Buffer.
2. Each vial can be used to carry out up to 50 reactions.
3. Dissolve vial contents when needed.
4. Store at -20°C. Use within two months.

5.8 TNB Standard

1. Dissolve in 1 mL of Assay Buffer XLI/ChE Assay Buffer to generate 2.5 mM TNB Standard.
2. Use within two months.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Using 2.5 mM solution of TNB, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	TNB Standard (µL)	Assay Buffer XLI/ChE Assay Buffer (µL)	Final volume standard in well (µL)	End amount of TNB standard in well (nmol/well)
1	0	400	400	0
2	4	396	400	5
3	8	392	400	10
4	12	388	400	15
5	16	384	400	20
6	20	380	400	25
7	24	376	400	30

Each dilution has enough standard to set up duplicate readings (2 x 200 µL).

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Blood:

1. Prepare a 40-200-fold dilution of Blood in dH_2O.
2. Record Dilution Factor.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 BChE inhibitor:

1. Dilute BChE Inhibitor 15-fold (i.e. Dilute 10 µL BChE Inhibitor with 140 µL Assay Buffer XLI/ChE Assay Buffer).

8.2 Blood sample:

1. Add 10-20 µl of Diluted Blood into 3 parallel well(s) assigned as $Sample_{AChE}$, $Sample_{BChE}$ and $Sample_{control}$, respectively.
2. Add 20 µL of Diluted BChE Inhibitor into the sample well assigned as $Sample_{AChE}$.
3. Add 20 µL of Assay Buffer XLI/ChE Assay Buffer into the other 2 sample well(s) assigned as $Sample_{BChE}$ and $Sample_{control}$.
4. These experimental conditions will lead to direct estimation of AChE Activity.
5. Adjust the volume to 95 µL/well with Assay Buffer XLI/ChE Assay Buffer.

8.3 AChE Positive Control:

1. Dilute AChE Positive Control/Acetylcholinesterase solution 50-fold in Assay Buffer XLI/ChE Assay Buffer.
2. Add 8-12 µL of Diluted AChE Positive Control/Acetylcholinesterase into desired well(s) assigned as AChE Positive Control.
3. Adjust the volume to 95 µL/well with Assay Buffer XLI/ChE Assay Buffer.

8.4 BChE Positive Control and BChE Inhibitor Positive Control:

1. Dilute BChE Positive Control/Butyrylcholinesterase solution 50-fold in Assay Buffer XLI/ChE Assay Buffer.
2. Add 8-12 µL of Diluted BChE Positive Control/Butyrylcholinesterase into 2 separate wells.

3. Add 20 µL of Diluted BChE Inhibitor into one well, assigned as BChE Inhibitor Positive Control and add 20 µL of Assay Buffer XLI/ChE assay buffer into the other well assigned as BChE Positive Control.
4. Adjust the volume of $Sample_{AChE,}$ $Sample_{BChE,}$ $Sample_{control}$ AChE Positive Control, BChE Positive Control, and BChE Inhibitor Positive Control to 95 µL/well with Assay Buffer XLI/ChE Assay Buffer.

Δ Note: 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.

Δ Note: 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 presence of final solvent concentration).

8.5 DTNB:

1. Add 5 µL DTNB solution to each well containing $Sample_{AChE}$, $Sample_{BChE}$, $Sample_{control}$, AChE Positive Control, BChE Positive Control and BChE Inhibitor Positive Control.
2. The total volume in every well (i.e. samples, background controls, positive controls) should be 100 µL.
3. Incubate plate for 10 minutes at room temperature with gentle shaking, protect from light.

8.6 AChE and BChE substrate preparation:

1. Prepare a 120-fold dilution of AChE and a 120-fold dilution of BChE substrate, respectively (i.e. Dilute 5 µL of each substrate with 595 µL Assay Buffer XLI/ChE Assay Buffer), vortex briefly.
2. Add 100 µL of Diluted AChE substrate to wells containing $Blood_{AChE}$, AchE Positive Control.
3. Add 100 µL of Diluted BChE substrate to wells containing $Blood_{BChE}$, BChE Positive Control and BChE Inhibitor Positive Control.

4. Add 100 µL of Assay Buffer XLI/ChE Assay Buffer to well assigned as $Blood_{control}$.
5. Mix well.

8.7 Measurement:

1. Measure absorbance immediately at 412 nm in kinetic mode for 10-30 minutes at room temperature.
2. 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).
3. The TNB Standard Curve (see Standard Preparation) can be read in Endpoint mode.

9. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. Plot the TNB Standard Curve.
4. Apply the ΔOD to the TNB Standard Curve to get B nmol of TNB generated during the reaction time ($\Delta t = t_2 - t_1$).
5. Subtract the sample background control reading from its paired sample reading ($B_{\text{test sample}} - B_{\text{sample background control}}$)/Δt.

$$\text{Sample}\frac{\text{AChE}}{\text{BChE}}\text{Activity} = \frac{\text{(B sample (AChE or BChe) - B sample (control))}}{\Delta t M} * D$$

$$= (\text{nmol/min})/\text{mL} = \text{mU/mL}$$

Where:

B = Amount of TNB in the sample well (nmol).

Δt = 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 minute at pH 7.5 at room temperature.

10. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

11. Typical Data

Data provided **for demonstration purposes only.**

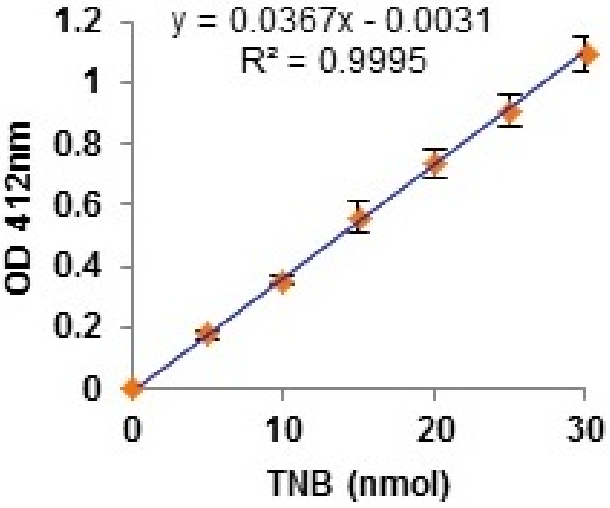

Figure 1. TNB Standard Curve.

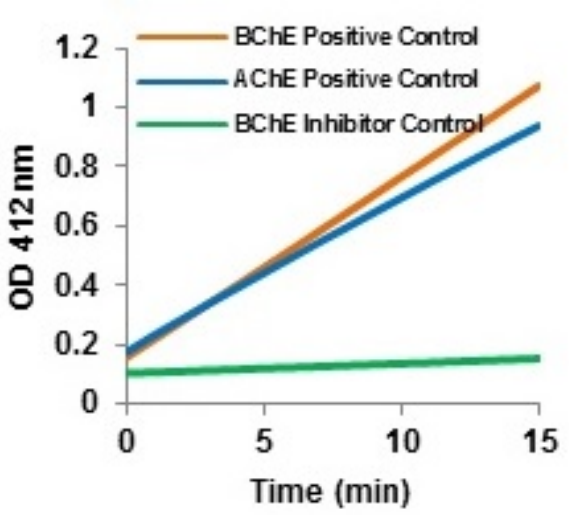

Figure 2. Measurement of purified BChE activity with or without BChE inhibitor and purified AChE activity.

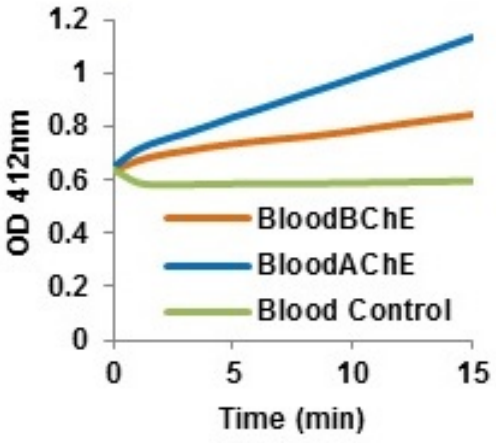

Figure 3. AChE and BChE activity in Human Blood (10 µlL 1:50 dilution).

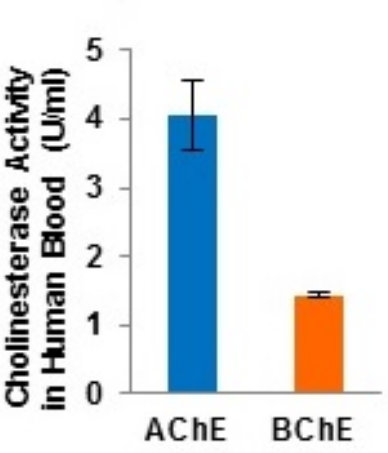

Figure 3. AChE and BChE activity in Human Blood (10 µlL 1:50 dilution).

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)