CREATIVE BIOMART®
Assay Kit

Cholinesterase Activity Assay Kit (Colorimetric)

Product Information

Cat

Kit-1070

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Description

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. 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.

Applications

Measurement of ChE activity in various tissues/cells
Screening of ChE inhibitors

Storage

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Store Kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.
ChE Assay Buffer: Store at 4 °C or -20 °C. Bring to room temperature before use.
AChE Substrate: Reconstitute in 100 µl ChE Assay Buffer. Store at -20°C. Use within two months.
BChE Substrate: Store at -20°C, protect from light. Bring to room temperature before use.
Acetylcholinesterase: Reconstitute with 100 µl of ChE Assay Buffer. Aliquot and store at -20 °C. Use within two months.
Butyrylcholinesterase: Reconstitute with 20 µl ChE Assay Buffer. Store at -20 °C. Use within two months.
BChE Inhibitor: Reconstitute BChE Inhibitor in 150 µl dH2O. Vortex intensively at room temperature to facilitate solubilization. Aliquot and store at -20°C. Bring to room temperature before use. Use it within two months.
DTNB Solution: Dissolve 1 vial of DTNB with 625 µl ChE Assay Buffer. Each vial can be used to carry out up to 50 reactions. Dissolve
vial contents when needed. Store at -20°C. Use within two months.
TNB Standard: Dissolve in 1 ml of ChE Assay Buffer to generate 2.5 mM TNB Standard. Use within two months.

Shipping

Gel Pack

Size

100 assays

Kit Components

AChE Assay Buffer;
AChE Substrate;
BChE Probe (in DMSO);
Acetylcholinesterase;
Butyrylcholinesterase;
BChE Inhibitor;
DTNB;

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TNB Standard

Materials Required but Not Supplied

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

Target Species

Mammalian

Detection method Absorbance (OD 412 nm)

Compatible Sample Types

Biological Fluids: Blood

Features & Benefits

Rapid, simple & convenient;
This assay kit can detect cholinesterase activity as low as 0.5 mU/ml in a variety of samples

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 dH2O. Record Dilution Factor. Add 10-20 µl of Diluted Blood into 3 parallel well(s) assigned as BloodAChE, BloodBChE and Bloodcontrol, respectively; Add 20 µl of Diluted BChE Inhibitor into the sample well assigned as BloodAChE; add 20 µl of ChE Assay Buffer into the other 2 sample well(s) assigned as BloodBChE and Bloodcontrol. These experimental conditions will lead to direct estimation of AChE Activity.
c. For AChE Positive Control: Dilute Acetylcholinesterase solution 50-fold in ChE Assay Buffer. Add 8-12 µl of Diluted Acetylcholinesterase into desired well(s) assigned as AChE Positive Control.
d. For BChE Positive Control and BChE Inhibitor Positive Control: Dilute 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

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sample(s), background control(s) and Positive Control (AChE and BChE) 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 presence of final solvent concentration).
2. TNB Standard Curve: Add 0, 2, 4, 6, 8, 10, 12 µl of the 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 ChE Assay Buffer.
3. DTNB: Add 5 µl DTNB solution to each well containing BloodAChE, BloodBChE, Bloodcontrol, AChE, BChE and BChE Inhibitor Positive Control. The total volume in every well (i.e. samples, background controls, positive controls) should be 100 µl. Incubate plate for 10 min at room temperature with gentle shaking, protect from light.
4. AChE and BChE substrate preparation: 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 ChE Assay Buffer), vortex briefly. Add 100 µl of Diluted AChE substrate to wells containing BloodAChE, AchE Positive Control. Add 100 µl of Diluted BChE substrate to wells containing BloodBChE, BChE Positive Control and BChE Inhibitor Positive Control; Add 100 µl of ChE Assay Buffer to well assigned as Bloodcontrol, Mix well.
5. Measurement: Measure absorbance immediately at 412 nm in kinetic mode for 10-30 min at room temperature. Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding absorbance values (OD1 and OD2). The TNB Standard Curve (see step 2) can be read in Endpoint mode.
Note: We suggest carefully shake the microplate for 10 seconds to mix contents prior to start of read-out.

Analysis

6. Calculation: Subtract 0 Standard reading from all readings. Plot the TNB Standard Curve. Calculate the AChE and BChE activity of the test sample: ΔOD = OD2 – OD1. Apply the ΔOD to the TNB Standard Curve to get B nmol of TNB generated during the reaction time (Δt = t2 - t1). Subtract the sample background control reading from its paired sample reading (B test sample-B sample

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background control)/Δt.
Sample AChE/BChE Activity =(B test sample-B sample control)/ (Δt*M) □ D = nmol/min/ml = mU/ml
Where: B = TNB amount from Standard Curve (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 min. at pH 7.5 at room temperature.

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