KAMIYA BIOMEDICAL COMPANY

Butyrylcholinesterase Fluorescent Activity Kit

For the determination of BChE activity in Serum and Plasma

Cat. No. KT-710

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION
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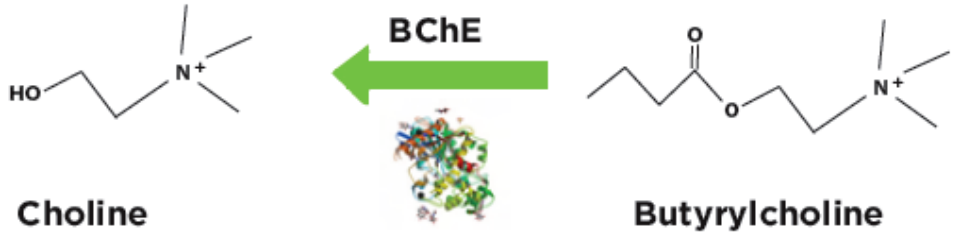
Background
Butyrylcholinesterase (BChE) belongs to the same structural class of proteins as acetylcholinesterase (AChE). The 440kDa tetrameric glycoprotein is predominantly found in blood, kidneys, intestine, liver, lung, heart and the central nervous system. Many species, such as human, horse and mice exhibit high BChE activity in plasma, whereas rats have higher acetylcholinesterase activity in plasma. BChE preferentially acts on butyrylcholine, but also hydrolyzes acetylcholine.



BChE serves a few known functions within the body. As a detoxification enzyme, it hydrolyzes ester-containing drugs and scavenges cholinesterase inhibitors, such as succinylcholine, before they have a chance to reach synaptic targets. By doing this, the enzyme minimizes the neuromuscular effect these agents have. A deficiency of BChE can result in delayed metabolism of various drugs, such as cocaine, and treatment with doses of BChE can help in overcoming the physiological reaction to them. As an activator enzyme, BChE converts administered prodrugs into functional therapeutics. Bambuterol is a prodrug with anti-asthmatic properties after being converted by BChE. BChE is the only enzyme in human serum that acts on heroin, and its end product, after crossing the blood-brain barrier, is hydrolyzed to morphine by enzymes in the brain.

Alzheimer's disease involves the degeneration of cholinergic neurons and loss of cholinergic transmission. The reduction in choline acetyltransferase leads to a decrease in acetylcholine and acetylcholinesterase activity, which appears to cause an increase in BChE activity. Potent cholinesterase inhibitor therapeutics protect the limited acetylcholine levels, acting on both AChE and BChE. Selective BChE inhibitors prevent the formation of new beta-amyloid plaques, which are created by BChE cleaving amyloid precursor protein to beta-amyloid protein. BChE-positive neurons project to the frontal cortex portion of the brain. BChE may have roles in attention, executive function, emotional memory and behaviour. As dementia advances, BChE activity has been shown to increase, while AChE activity decreases, leaving the potential for BChE activity to be used as a biomarker for progression or target for future therapies.

PRINCIPLE
The K-ASSAY® Butyrylcholinesterase Activity kit is designed to quantitatively measure butyrylcholinesterase (BChE) activity in a variety of samples. Please read the complete kit insert before performing this assay. A human BChE calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. The kit utilizes a proprietary non-fluorescent molecule, ThioStar, that covalently binds to the thiol product of the reaction between the BChE Substrate and BChE in the calibrators or samples, yielding a fluorescent product read at 510 nm in a fluorescent plate reader with excitation at 390 nm.

The kit is suitable for measuring BChE activity in appropriately diluted serum or plasma from a number of species. It will also measure BChE in extracted tissue samples and cell lysates. Because the readout of BChE activity is purely chemical, there are few interferants that will affect the readings obtained.

REACTION OVERVIEW
1. Sample or calibrator added to well.
2. The reaction is initiated with the addition of the Reaction Mix containing BChE Substrate and ThioStar Reagent.
3. Incubate for 20 minutes and read fluorescent signal. Calculate BChE activity from calibration curve.

4. Alternatively samples can be read kinetically. Follow steps 1 and 2 above. Add Reaction Mix and read signal at 510 nm over time. Compare rates for samples and calibrators to determine sample BChE activity.

COMPONENTS

- Black 96 Well Plates 2 Plates
- Butyrylcholinesterase Calibrator 225 µL
- ThioStar Detection Reagent 2 vials
- Dry DMSO 14 mL
- Assay Buffer Concentrate 28 mL
- BChE Substrate 2 vials

Storage

All components of this kit should be stored at 4°C until the expiration date of the kit.

DMSO, when stored at 4°C, will freeze. Can be stored tightly capped at room temperature.

Other Materials Required

- Distilled or deionized water.
- Repeater pipet with disposable tips capable of dispensing 50 µL.
- Fluorescence 96 well plate reader capable of reading fluorescent emission at 510 nm, with excitation at 390 nm. Contact your plate reader manufacturer for correct filter sets. Set plate parameters for a 96-well Corning Costar 3650 plate.
- Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals.

The Butyrylcholinesterase Calibrator is derived from human blood. It has been extensively tested for viral contamination, but all human blood products should be treated as potentially infectious and adequate precautions taken.

ThioStar Detection Reagent should be stored at 4°C in the desiccator. Allow to warm to room temperature prior to opening. ThioStar will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin and Kathon will react with the substrate.

Sample Types

This assay has been validated for serum and EDTA and heparin plasmas from a variety of species. Samples containing visible particulate should be centrifuged prior to using.

Sample Preparation

Serum & Plasma

Store separated serum or plasma on ice until assaying or freeze in aliquots for later use. Samples must be diluted in Assay Buffer prior to running in the kit. Any samples with BChE activity outside the calibration curve range should be diluted further with Assay Buffer to obtain readings within the calibration curve. Human serum and plasma typically have to be diluted ≥ 1:300 to read in the assay.

Use all samples within 2 hours of dilution.

Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all calibrators and samples be run in duplicate to allow the end user to accurately determine BChE activity. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Prepare the Assay Buffer by diluting one part of the 10x Assay Buffer Concentrate with nine parts deionized water for a 1:10 dilution. It is stable for up to 3 months when stored at 4°C.

ThioStar Detection Reagent

Remove a vial of ThioStar Reagent from the desiccator and add 700 µL of the provided DMSO to the vial. Vortex thoroughly. Store any unused reconstituted Detection Reagent at 4°C in the desiccator and use within 2 weeks.

Acetylcholinesterase Substrate

Add 700 µL of the provided DMSO to the BChE Substrate vial and vortex thoroughly. This is a 10x concentrate of the substrate. Store any unused reconstituted BChE Substrate at room temperature and use within 2 weeks.

Reaction Mix Dilution Table

	1/2 Plate	Full Plate
10X BChE Substrate Concentrate	300 µL	550 µL
10X ThioStar® Concentrate	300 µL	550 µL
DMSO	2.4 mL	4.4 mL

Calibrator Preparation

BChE Calibrators are prepared by labeling seven test tubes as #1 through #7. Briefly spin vial of calibrator in a microcentrifuge to ensure contents are at bottom of vial. Pipet 450 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #7. Carefully add 50 µL of the BChE Calibrator to tube #1 and vortex completely. Take 250 µL of the BChE solution in tube #1 and add it to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 through #7. The activity of BChE in tubes 1 through 7 will be 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 mU/mL.

Use all Calibrators within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	450	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	50	250	250	250	250	250	250
Final Conc. (mU/mL)	20	10	5	2.5	1.25	0.625	0.313

ASSAY PROTOCOL

1. Use the plate layout sheet on the last page of the insert to aid in proper sample and calibrator identification. Set plate parameters for a 96-well Corning Costar 3650 plate.

2. Pipet 100 µL of samples or calibrators into duplicate wells in the plate.
3. Pipet 100 µL of Assay Buffer into duplicate wells as a Zero calibrator.
4. Add 50 µL of the prepared Reaction Mix to each of the wells using a repeater pipet.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate at room temperature for 20 minutes.
7. Read the fluorescent emission at 510 nm with excitation at 370-410 nm. Please contact your plate reader manufacturer for suitable filter sets.

Calculation of Results

Average the duplicate FLU readings for each calibrator and sample. Create a calibration curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero calibrator. The sample activity obtained should be multiplied by the dilution factor to obtain neat sample values.

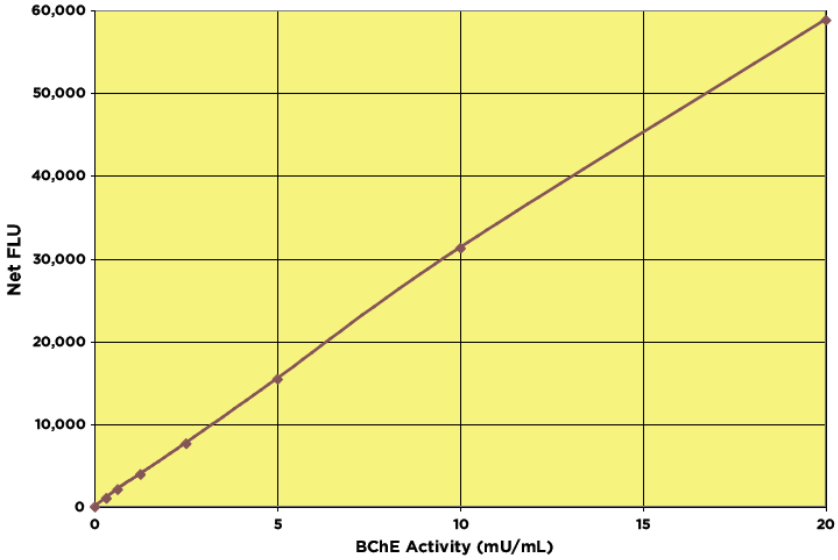
Typical Data

Always run your own calibration curve for calculation of results.

Sample	Mean FLU	Net FLU	BChE Activity (mU/mL)
Standard 1	59,868	58,814	20
Standard 2	32,329	31,275	10
Standard 3	16,480	15,426	5
Standard 4	8,706	7,652	2.5
Standard 5	4,988	3,934	1.25
Standard 6	3,136	2,082	0.625
Standard 7	2,093	1,039	0.313
Zero	1,054	0	0
Sample 1	26,418	25,364	8.10
Sample 2	5,979	4,925	1.62

Do not use this data.

Typical Calibration Curve



Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the FLUs for twenty wells run for each of the zero and calibrator #7. The detection limit was determined at two (2) standard deviations from the zero along the calibration curve.
Sensitivity was determined as 0.018 mU/mL.

The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the zero and a low activity plasma sample.
The Limit of Detection was determined as 0.012 mU/mL.

Intra Assay Precision

Three mammalian samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated BChE activities were:

Sample	BChE Activity (mU/mL)	%CV
1	5.70	4.7
2	2.97	7.3
3	1.17	7.5

Inter Assay Precision

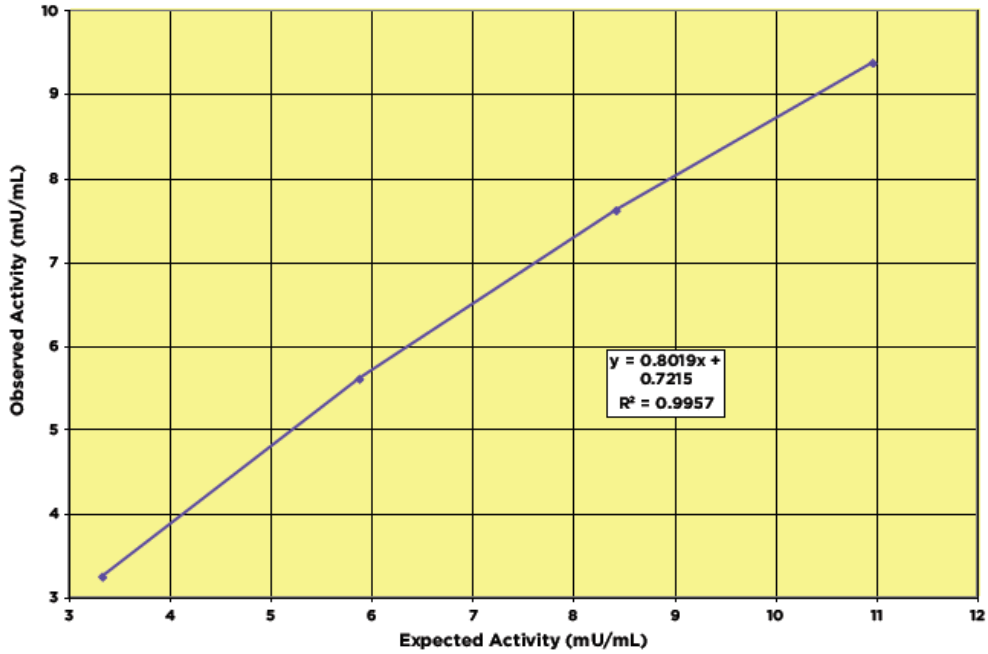
Three mammalian samples were diluted with Assay Buffer and run in duplicates in thirteen assays run over multiple days by four operators. The mean and precision of the calculated BChE activities were:

Sample	BChE Activity (mU/mL)	%CV
1	7.70	9.1
2	5.84	7.5
3	1.71	8.5

Linearity

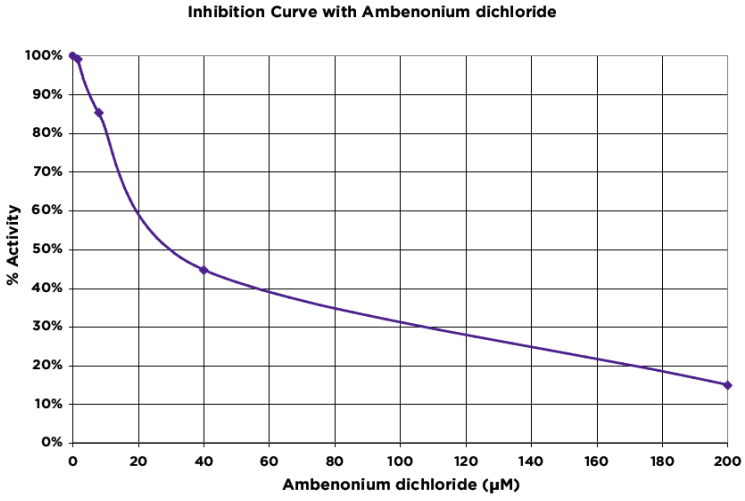
Linearity was determined by taking two serum samples, one high sample diluted 1:450 and one low sample diluted 1:450, and mixing in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

Low Sample	High Sample	Observed Activity (mU/mL)	Expected Activity (mU/mL)	% Recovery
100%	0%	0.79	---	---
80%	20%	3.24	3.33	97.2
60%	40%	5.60	5.88	95.3
40%	60%	7.61	8.42	90.4
20%	80%	9.37	10.97	85.4
0%	100%	13.51	---	---
			Mean Recovery	**92.1%**



Inhibition Studies

The human BChE calibrator was incubated with varying concentrations of a reversible inhibitor of BChE activity, Ambenonium dichloride, from 200 µM down to 16 µM for 19 hours at room temperature in the kit Assay Buffer. The activity in the incubated enzyme samples was then determined. in the normal manner by adding 100 µL of the samples and reading the activity after a 20 minute incubation with 50 µL of Reaction Mixture.



Sample Values

A variety of serum and plasma samples were tested in the assay, including chicken, mouse, rat, dog, monkey, pig and human samples. Values averaged 4,565 mU/mL. The average for 23 human serum and plasma samples was 6,268 ± 2,506 mU/mL. Five rat serum and plasma samples had low activity levels of between 293 and 365 mU/mL.

Cross Reactivity

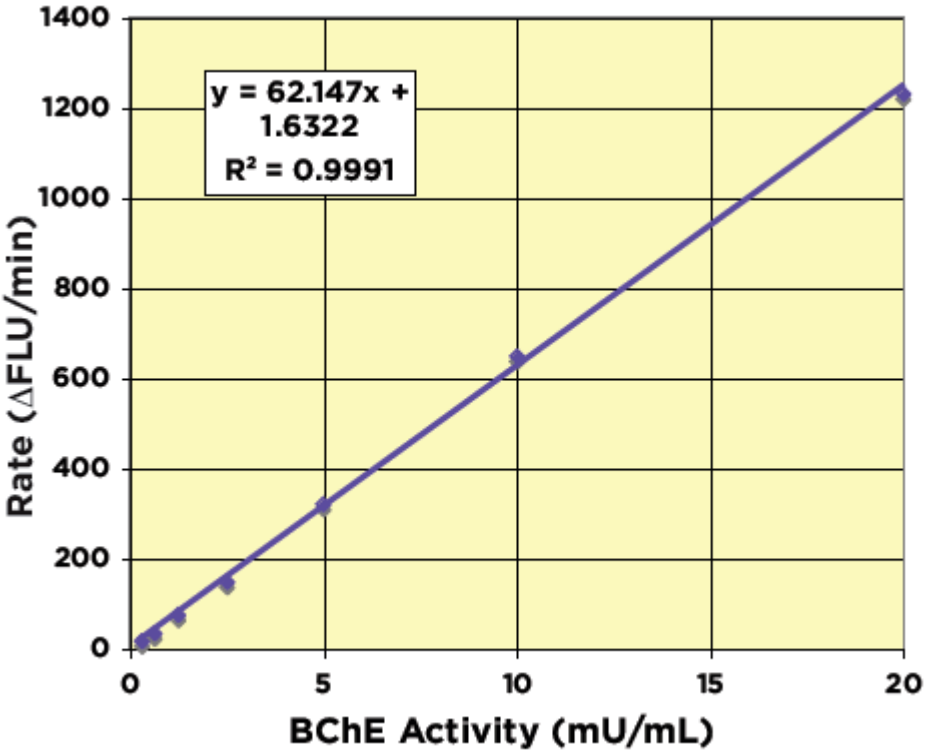
The assay was tested with a sample of human acetylcholinesterase from our Acetylcholinesterase Fluorescent Activity Assay, Catalog Number KT-708. The human AChE sample when used in the assay read at less than 0.2% of its expected activity.

Interferents

A variety of additives were tested as possible interfering substances in the assay. 1% ethanol in the well decreased the activity recorded by 12.6%, whereas 0.5% ethanol in the well decreased activity by almost 10.3%. 5% DMSO in the well increased activity by 0.2% and 1% DMF in the well increased activity by 6.5%. 10% methanol in the well increased activity by 0.6%. 1% Triton X-100 in the well increased activity 4.0% and 0.1% hemoglobin decreased activity 4.2%. Controls should be run by the end user when appropriate.

End Point Versus Kinetic Activity

A serum preparation diluted 1:900 read 11.89 mU/mL in Arbor Assays' endpoint assay. It was also read off a kinetic assessment of Butyrylcholinesterase activity and an activity of 12.37 mU/mL was obtained.



H	G	F	E	D	C	B	A	
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FOR RESEARCH USE ONLY

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