ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Acetylcholinesterase
Fluorescent Activity Kit

2 Plate Kit Catalog Number K015-F1

Species Independent

Sample Types Validated:

Serum, Plasma, and Erythrocyte Membranes

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

TABLE OF CONTENTS

BACKGROUND

Acetylcholinesterases (AChE) appear critical to both development and function of the nervous system. The use of AChE inhibitors as therapeutic agents and pesticides has spurred detailed investigations of cholinesterases since their identification by Dale[1]. Acetylcholine (ACh) is an essential neurotransmitter in the central and peripheral nervous systems. In the brain multiple areas exist where cholinergic neurons are concentrated[2]. Nicotinic and muscarinic ACh receptors are recognized as binding and effector proteins to mediate chemical neurotransmission at neurons, ganglia, heart and smooth muscle fibers and glands. This traditional view of AChE acting solely as neurotransmitter has to be revised based on the findings published both early and late in the last century, demonstrating the non-neuronal cholinergic system.

Choline **Acetylcholine**

Acetylcholinesterase is encoded by the single AChE gene; the structural diversity in the gene products arises from alternative nRNA splicing and post translational associations of catalytic and structural subunits. The major form of acetylcholinesterase found in brain, muscle, and other tissues is the hydrophilic species, which forms disulfide-linked oligomers with collagenous, lipid-containing structural subunits. The other alternatively-spliced form, expressed primarily in the erythroid tissues, is structurally different at the C-terminal end and contains a cleavable peptide with a GPI anchor. It associates with membrane receptors through phosphoinositide moieties added post-translationally[3]. Impairment of cholinergic neurotransmission is well-established in Alzheimer's disease, but there is controversy about its relevance at the early stages of the disease as well as in mild cognitive impairment. *In vivo* positron emission tomography imaging of cortical AChE activity as a marker of cholinergic function that is expressed by cholinergic axons and neurons has demonstrated a reduction of this enzyme activity in manifest Alzheimer's patients[4]. Other intentional or environmental methods of impairment is with organophosphates and carbamates with anticholinergic properties which are used as insecticides worldwide or as warfare agents. Thousands of cases of acute poisoning have been reported[5]. This acute toxicity inhibits AChE at nerve terminals where inhibition causes accumulation of ACh. This, in turn, induces overstimulation of nicotinic and muscarinic receptors in the central and peripheral nervous systems and the consequent signs and symptoms[6].

1. Dale, H. H. (1914). The action of certain esters and ethers of choline, and their relation to muscarine. *Journal of Pharmacology and Experimental Therapeutics*, 6(2), 147–190.
2. Perry, E., et al. (1999). Acetylcholine in mind: a neurotransmitter correlate of consciousness? *Trends in Neurosciences*, 22(6), 273–280.
3. NCBI (n.d.). ACHE acetylcholinesterase (Cartwright blood group) [Homo sapiens (humans)]. Retrieved from https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=43.
4. Herholz, K. (2008). Acetylcholine esterase activity in mild cognitive impairment and Alzheimer's disease. E*uropean Journal of Nuclear Medicine and Molecular Imaging*, 35(1), 25–29.
5. Moretto, A., & Lotti, M. (2004). Toxicity of pesticides. In N. H. Stacey (Ed.). *Occupational Toxicology*, 177–204. London, GB: Taylor & Francis.
6. Lotti, M. (1991). Treatment of acute organophosphate poisoning. *Medical Journal of Australia*, 154(1), 51–55.

ASSAY PRINCIPLE

The DetectX® Acetylcholinesterase Activity Kit is designed to quantitatively measure acetylcholinesterase (AChE) activity in a variety of samples. Please read the complete kit insert before performing this assay. A human AChE standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a proprietary non-fluorescent molecule, ThioStar®, that covalently binds to the thiol product of the reaction between the AChE Substrate and AChE in the standards 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 AchE activity in appropriately diluted serum, plasma and RBC ghosts from a number of species. It will also measure AChE in extracted tissue samples and cell lysates. Because the readout of AChE activity is purely chemical, there are few interferants that will affect the readings obtained.

REACTION OVERVIEW

1. Sample or standard added to well.
2. The reaction is initiated with the addition of the Reaction Mix containing AChE Substrate and ThioStar® Reagent.
3. Incubate for 20 minutes and read fluorescent signal. Calculate AChE activity from standard 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 standards to determine sample AChE activity.

RELATED PRODUCTS

Kits	Catalog No.
Butyrylcholinesterase Fluorescent Activity Kit	K016-F1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione S-Transferase Activity Kit	K008-F1
Hemoglobin Colorimetric Detection Kit	K013-H1
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-H1X/K013-HX5
Histone Demethylase Fluorescent Activity Kit	K010-F1
P450 Fluorescent Activity Kit	K011-F1

SUPPLIED COMPONENTS

Black 96 Well Plates

See: www.ArborAssays.com/resources/#general-info for plate dimension data.

2 Plates Catalog Number X001-2EA

Acetylcholinesterase Standard

Recombinant Acetylcholinesterase (AChE) at 1,000 mU/mL in a special stabilizing solution.

225 µL Catalog Number C046-225UL

ThioStar® Detection Reagent

ThioStar thiol detection substrate stored in a ziploc pouch with desiccant. Reconstitute with dry DMSO.

2 vials Catalog Number C048-1EA

Dry DMSO

Dry Dimethyl sulfoxide solvent over molecular sieves. **May be stored at room temperature.**

14 mL Catalog Number X022-14ML

Assay Buffer Concentrate

A 10x concentrated Tris buffer containing detergents and stabilizers.

28 mL Catalog Number X064-28ML

AChE Substrate

Acetylthiocholine iodide freeze dried with stabilizers.

2 vials Catalog Number C047-1EA

STORAGE INSTRUCTIONS

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. It 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 3915 plate. See: www.ArborAssays.com/resources/#general-info

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.

ThioStar® Detection Reagent should be stored at 4°C in the desiccated pouch. 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.

Reconstituted ThioStar in DMSO stored at 4°C in the supplied desiccated pouch can be used up to 2 months later. The background on the reconstituted ThioStar will increase slowly over time but the increase will not affect the assay results obtained.

SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasma, and solubilized RBC ghosts from a variety of species. Samples containing visible particulate should be centrifuged prior to using. All samples and buffers should be free of excess thiols and reducing agents such as ß-mercaptoethanol, TCEP, or DTT.

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 AChE activity outside the standard curve range should be diluted further with Assay Buffer to obtain readings within the standard curve. Serum and plasma typically have to be diluted ≥ 1:300 to read in the assay.

Erythrocytes (RBCs)

Blood is collected in the presence of heparin or EDTA. The sample is then centrifuged and the plasma and white cell layer are removed from the RBC layer. The RBCs are suspended and gently washed twice with three volumes of isotonic saline (0.9%), separating the cells by centrifugation at 600 x g for 10 minutes and discarding the saline after each step. To lyse the RBCs, four volumes of cold deionized water are added to the RBCs. The cells are then vortexed and incubated for 10 minutes at 4˚C or allowed to undergo a freeze/thaw. Samples are centrifuged at 14,000 rpm for 10 minutes at 4˚C and the supernatant discarded.

Further wash the membrane pellet with two or three volumes of isotonic saline, with centrifugation in between, until it is only slightly pink. The smaller dark red pellet on the bottom is non-lysed RBCs and should be avoided. Solubilize the white membrane ghost pellet with Triton X-100. Final assay dilution of the solubilized RBC ghost sample must be sufficient that the assay sample contains ≤ 0.01% Triton X-100.

Use all samples within 2 hours of dilution.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. 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.

REAGENT PREPARATION CONTINUED

ThioStar® Detection Reagent

Allow the ziploc pouch to warm **completely** to room temperature prior to opening and remove a vial of ThioStar Reagent. Add 700 µL of the provided DMSO to the vial and vortex thoroughly. Store any unused reconstituted Detection Reagent at 4°C in the desiccated pouch and use within 2 months.

Acetylcholinesterase Substrate

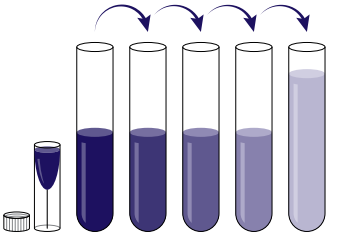
Add 700 µL of the provided DMSO to the AChE Substrate vial and vortex thoroughly. This is a 10x concentrate of the substrate. Store any unused reconstituted AChE Substrate at room temperature and use within 2 months.

Reaction Mix Dilution Table

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

Standard Preparation

AChE Standards are prepared by labeling test tubes as #1 through #5. Briefly spin vial of standard 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 #5. Carefully add 50 µL of the AChE Standard to tube #1 and vortex completely. Take 250 µL of the AChE solution in tube #1 and add it to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 through #5. The activity of AChE in tubes 1 through 5 will be 100, 50, 25, 12.5, and 6.25 mU/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5
Assay Buffer Volume (µL)	**450**	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4
Volume of Addition (µL)	**50**	250	250	250	250
Final Conc. (mU/mL)	100	50	25	12.5	6.25

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine AChE activity.

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3915 plate.
   See: www.ArborAssays.com/resources/#general-info for plate dimension data.
2. Pipet 100 µL of samples or standards into duplicate wells in the plate.
3. Pipet 100 µL of Assay Buffer into duplicate wells as a Zero standard.
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 standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard. The sample activity obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-acetylcholinesterase-fluorescent-activity-kit.assay

AChE Unit Definition

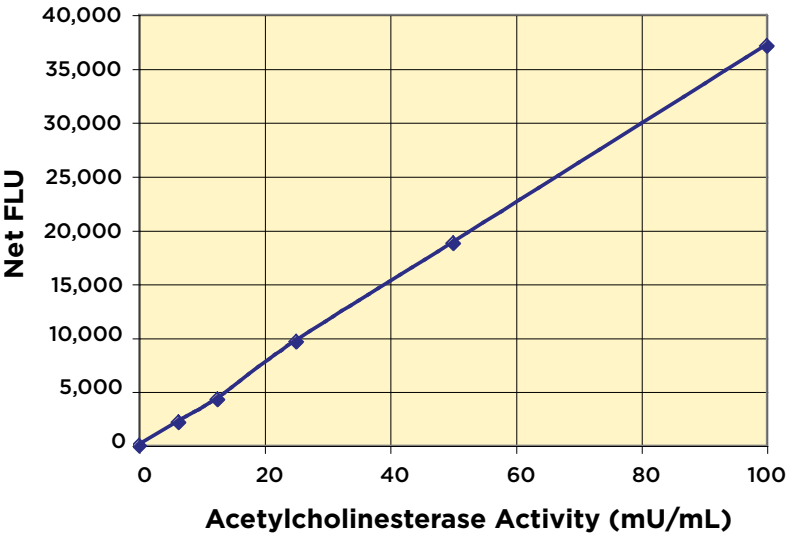
One unit will hydrolyze 1.0 µmol of acetylthiocholine iodide per minute at pH 7.4 and 37˚C.

TYPICAL DATA

Sample	Mean FLU	Net FLU	AChE Activity (mU/mL)
Standard 1	39,677	37,116	100
Standard 2	21,336	18,775	50
Standard 3	12,194	9,633	25
Standard 4	6,845	4,284	12.5
Standard 5	4,714	2,153	6.25
Zero	2,561	0	0
Sample 1	5,108	2,547	7.4
Sample 2	18,287	15,726	41.6

Always run your own standard curve for calculation of results. Do not use this data.

Typical Standard Curve



VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the FLUs for twenty wells run for each of the zero and standard #5. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.218 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 0.321 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 AChE activities were:

Sample	AChE Activity. (mU/mL)	%CV
1	63.9	8.9
2	41.6	9.1
3	11.5	6.7

Inter Assay Precision

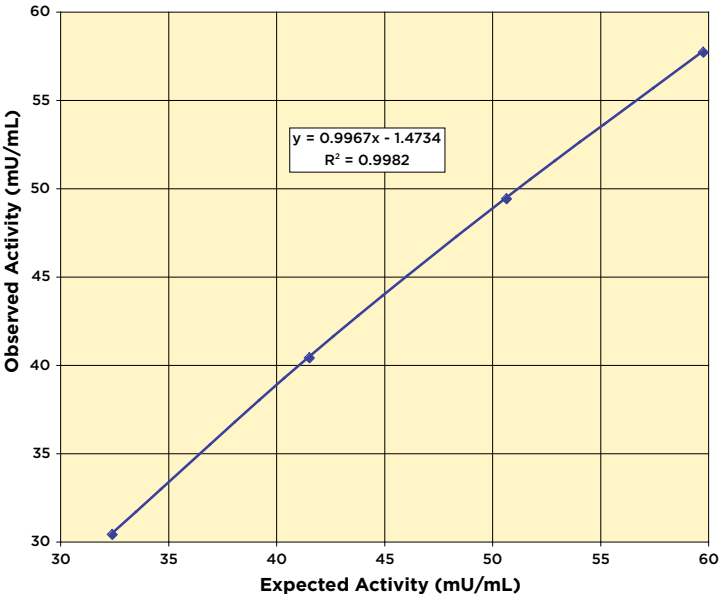
Three mammalian samples were diluted with Assay Buffer and run in duplicates in thirteen assays run over multiple days by four operators. The mean and precision of the calculated AChE activities were:

Sample	AChE Activity. (mU/mL)	%CV
1	59.1	7.9
2	39.9	8.5
3	11.3	11.1

Linearity

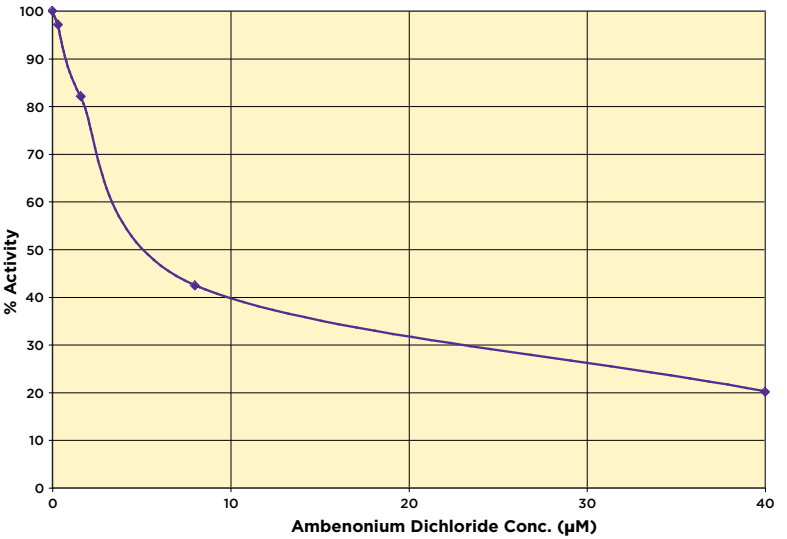
Linearity was determined by taking two serum samples, one high sample diluted 1:600 and one low sample diluted 1:1,000, and mixing in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

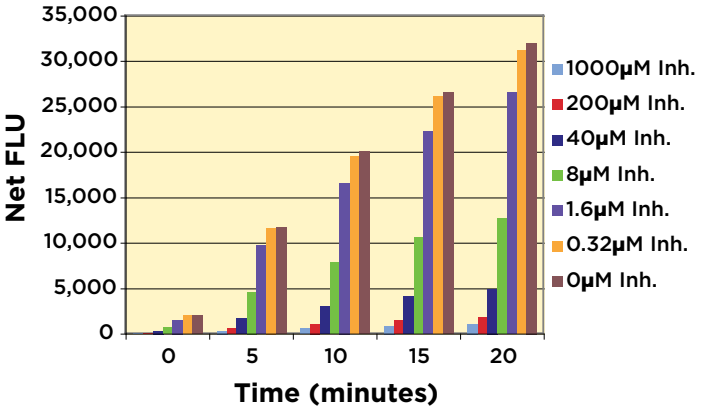
Low Sample	High Sample	Expected Activity (mU/mL)	Observed Activity (mU/mL)	% Recovery
80%	20%	32.4	30.4	93.8
60%	40%	41.5	40.4	97.3
40%	60%	50.7	49.4	97.5
20%	80%	59.8	57.7	96.5
			Mean Recovery	**96.3%**



Inhibition Studies

The human AChE standard was incubated with varying concentrations of a reversible inhibitor of AChE activity, Ambenonium dichloride, from 1,000 down to 0.32 µM for 17 hours at room temperature in the kit Assay Buffer. The activity in the incubated samples was then determined in the normal manner by adding 100 µL of the samples and kinetically reading the activity over 20 minutes.





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 19,188 mU/mL. RBC ghost samples ranged from 6,216 to 18,552 mU/mL with an average of 13,158 mU/mL.

CROSS REACTIVITY

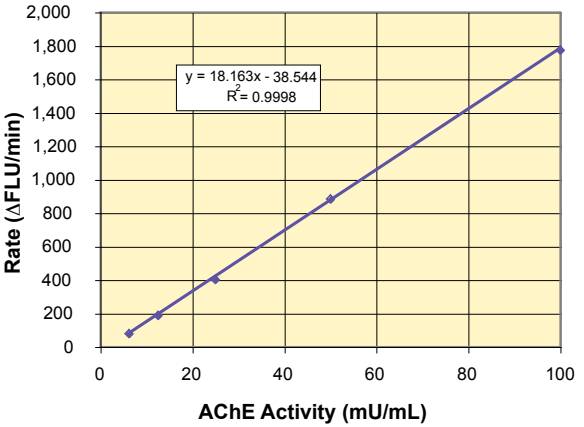
A sample of native human butyrylcholinesterase at 20 mU/mL was tested in the assay. It read 2.45 times higher than the same concentration of AChE tested at the same time.

INTERFERENTS

A variety of solvents were tested as possible interfering substances in the assay. Ethanol at 5% in the well increased the activity recorded by 6.4%, whereas at 10% in the well decreased activity by almost 43%. DMSO or DMF at 5% in the well decreased activity by 12.6% and 19.65% respectively. Methanol at 10% in the well decreased activity by 6.3%. We expect solvent levels at 1% of well volume to have little or no effect on the measured activity. A solvent only control should be run by the end user when appropriate.

END POINT VERSUS KINETIC ACTIVITY

Human RBC ghosts were read in a standard end point assay and in a kinetic assay. The value obtained from the end point assay was 12,577 mU/mL and in the kinetic assay, it read 12,453 mU/mL.



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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