

Fluoro BChE

Fluorescent Butyrylcholinesterase Detection Kit

Contact Information

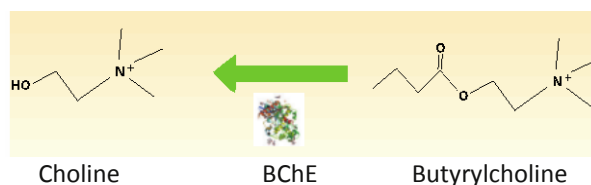
Address	Cell Technology Inc 48820 Kato Road Suite 400B Fremont, CA 94538 USA
Telephone	650-960-2170
Toll Free	888 7ASSAYS (727-7297)
Fax	650-960-0367
General Information	info@celltechnology.com
Sales	sales@celltechnology.com
Technical Questions	techsupport@celltechnology.com
Website	www.celltechnology.com

Notes:

Notes:	1
I. Introduction:	3
II. Assay Principle:	3
III. Storage:.....	4
IV. Schematic Representation of the BChE Fluorimetric Assay:	4
V. Warnings and Precautions:	4
VI. Catalog # FLBChE 100-2 contents and Storage (for 100 assays):.....	4
VII. Materials required but not supplied:.....	5
VIII. Sample types:.....	5
IX. Reagent preparation:.....	5
X. Standard Preparation:.....	6
XI. Assay Protocol:	6
XII. Calculation of Results:	6
XIII. Validation data:.....	7
XIV. Inhibition Studies:.....	9
XV. Interferents:	10
XVI. Endpoint versus Kinetic Activity:	10
XVII. References:.....	11

I. Introduction:

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 plasma¹. 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 them². 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 activity³. 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 protein⁴. 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 therapies⁵.

II. Assay Principle:

The Fluoro 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 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 non-fluorescent molecule, the Thiol Detection Reagent, that covalently binds to the thiol product of the reaction between the BChE Substrate and BChE 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 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.

III. 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.

IV. Schematic Representation of the BChE Fluorimetric Assay:

1. Sample or standard added to well.
2. The reaction is initiated with the addition of the Reaction Mix containing BChE Substrate and Thiol Detection Reagent.
3. Incubate for 20 minutes and read fluorescent signal. Calculate BChE 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 BChE activity.

V. Warnings and Precautions:

1. **For Research use only. Not for use in diagnostic procedures.**
2. Practice safe laboratory procedures by wearing gloves, protective clothing and eyewear.
3. DMSO is a powerful aprotic organic solvent that enhances the rate of absorption of skin permeable substances. Wear protective gloves when handling the solvent.
4. The Butyrylcholinesterase standard is derived from human blood, and has been extensively tested for viral contamination. However, adequate precautions should be taken as all human blood products are treated as potentially infectious.
5. The Thiol Detection reagent should be stored at 4°C in the desiccator. Buffers containing preservatives such as sodium azide, Proclin and Kathon will react with it.

VI. Catalog # FLBChE 100-2 contents and Storage (for 100 assays):

- | | | |
|---|----------|----------------------|
| 1. Black 96 Well Plates (Costar 3650) | 2 Plates | Catalog Number 90047 |
| 2. Butyrylcholinesterase Standard
Butyrylcholinesterase (BChE) at 200 mU/mL in a special stabilizing solution. CAUTION: This material is isolated from human serum. Treat as potentially infectious. | 225 µL | Catalog Number 90042 |
| 3. Thiol Detection Reagent
Thiol detection substrate stored in a desiccator. Reconstitute with dry DMSO. | 2 vials | Catalog Number 90043 |
| 4. BChE Substrate
Butyrylthiocholine iodide freeze dried with stabilizers. | 2 vials | Catalog Number 90044 |
| 5. Anhydrous DMSO
Dry Dimethyl sulfoxide solvent over molecular sieves. May be stored at room temperature. | 14 mL | Catalog Number 90045 |
| 6. Assay Buffer Concentrate
A 10x concentrated Tris buffer containing detergents and stabilizers. | 28 mL | Catalog Number 90046 |

VII. Materials required but not supplied:

1. Deionized or Distilled water
2. 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.
3. 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.

VIII. 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 standard curve range should be diluted further with Assay Buffer to obtain readings within the standard curve. Human serum and plasma typically have to be diluted $\geq 1:300$ to read in the assay. Use all samples within 2 hours of dilution.

IX. Reagent preparation:

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards 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.

1. 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.

2. Thiol Detection Reagent:

Remove a vial of Thiol Reagent from the desiccator and add 700 μL of the provided DMSO to the vial. Vortex thoroughly. This is a 10X concentrate of the Thiol Detection Reagent. Store any unused reconstituted Detection Reagent at 4°C in the desiccator and use within 2 weeks.

3. Butyrylcholinesterase 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:

	½ plate	Full plate
10X BChE Substrate Concentrate	300 μL	550 μL
10X Thiol Detection Reagent Concentrate	300 μL	550 μL
DMSO	2.4mL	4.4mL

X. Standard Preparation:

BChE Standards are prepared by labeling seven test tubes as #1 through #7. Briefly spin vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 450 μ L of Assay Buffer into tube #1 and 250 μ L into tubes #2 to #7. Carefully add 50 μ L of the BChE Standard to tube #1 and vortex completely. Take 250 μ L 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 Standards 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

XI. Assay Protocol:

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 3650 plate.
2. Pipet 100 μ L of samples or standards into duplicate wells in the plate.
3. Pipet 100 μ L of Assay Buffer into duplicate wells as a Zero standard.
4. Add 50 μ L 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.

XII. Calculation of Results:

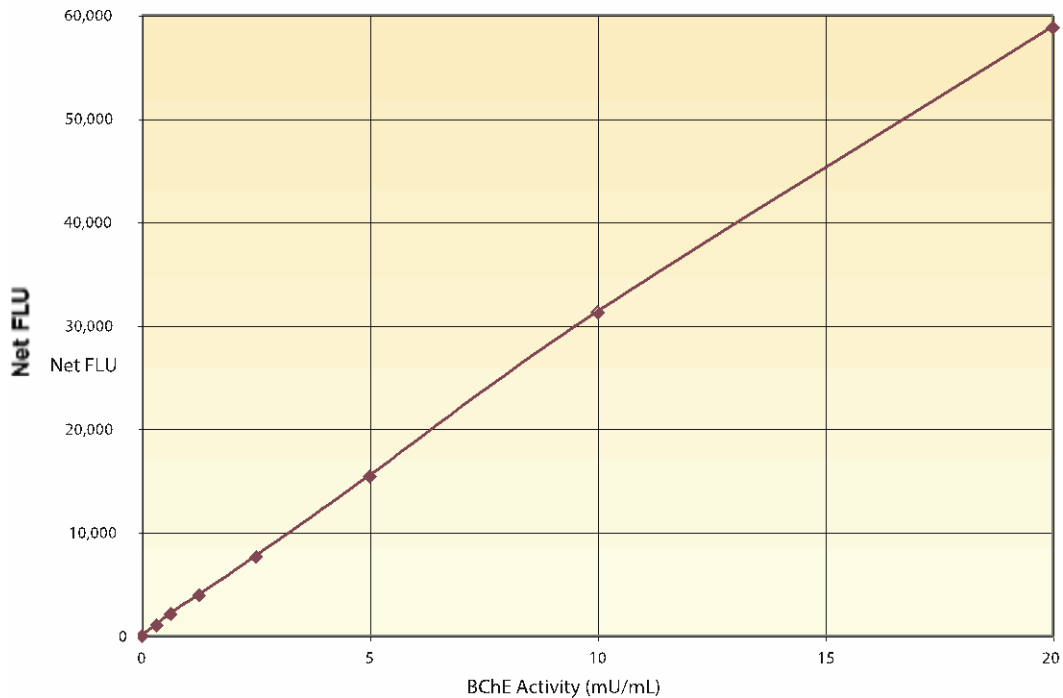
Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard. The sample activity obtained should be multiplied by the dilution factor to obtain neat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

TYPICAL DATA

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

Typical Standard Curve



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

XIII. 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 #7. The detection limit was determined at two (2) standard deviations from the zero along the standard 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 EDTA plasma sample. The Limit of Detection was determined as 0.012 mU/mL.

Intra Assay Precision:

Three human serum 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

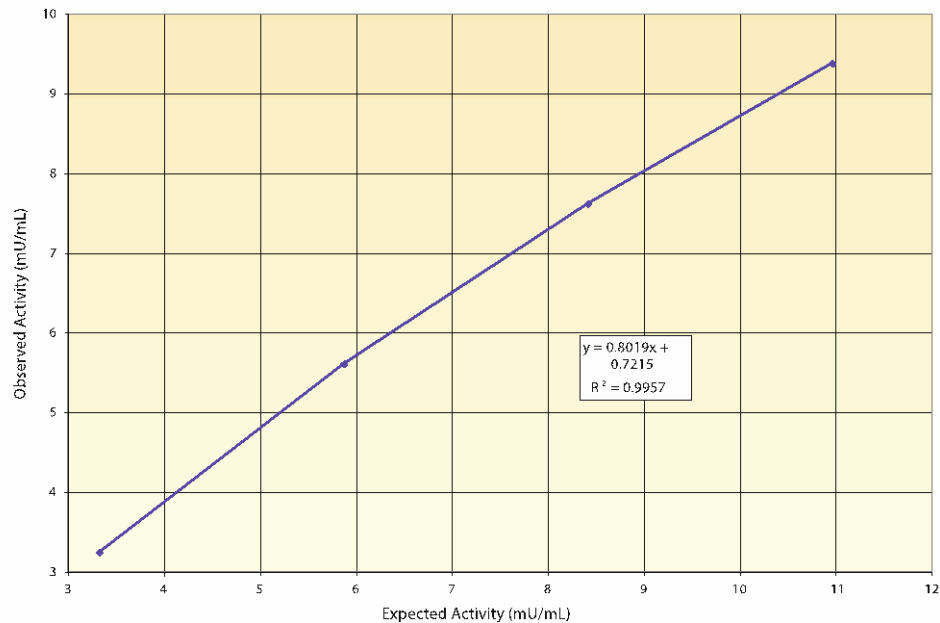
Inter Assay Precision:

Three human serum samples were diluted with Assay Buffer and run in duplicates in sixteen 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 was determined by taking two plasma 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%



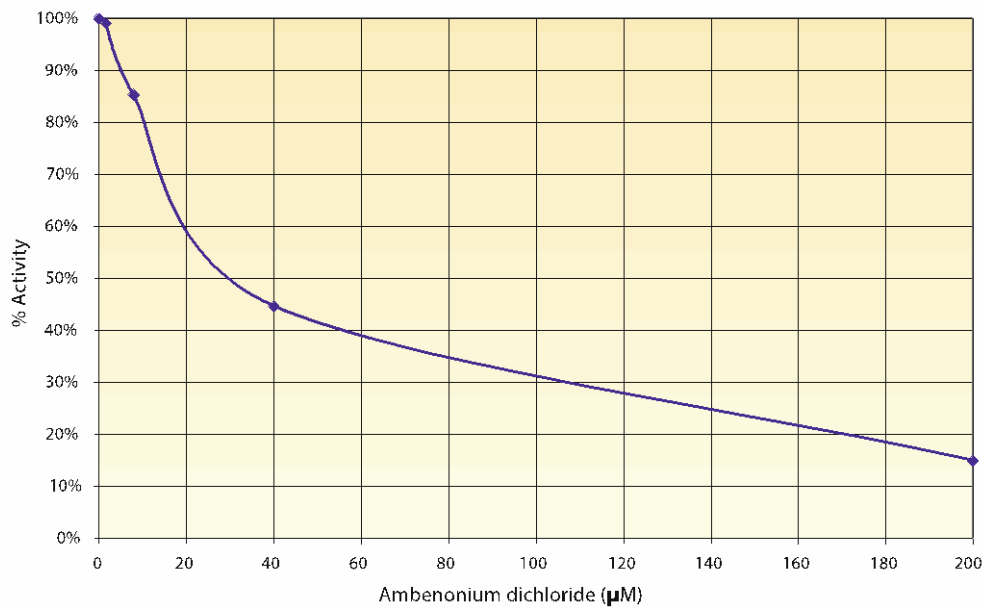
XIV. Inhibition Studies:

The human BChE standard was incubated with varying concentrations of a reversible inhibitor of BChE activity, Ambenonium dichloride, from 200 μ M down to 16 μ M 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 μ L of the samples and reading the activity after a 20 minute incubation with 50 μ L 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 $6,268 \pm 2,506$ mU/mL. Five rat serum and plasma samples had low activity levels of between 293 and 365 mU/mL.

Inhibition Curve with Ambenonium dichloride

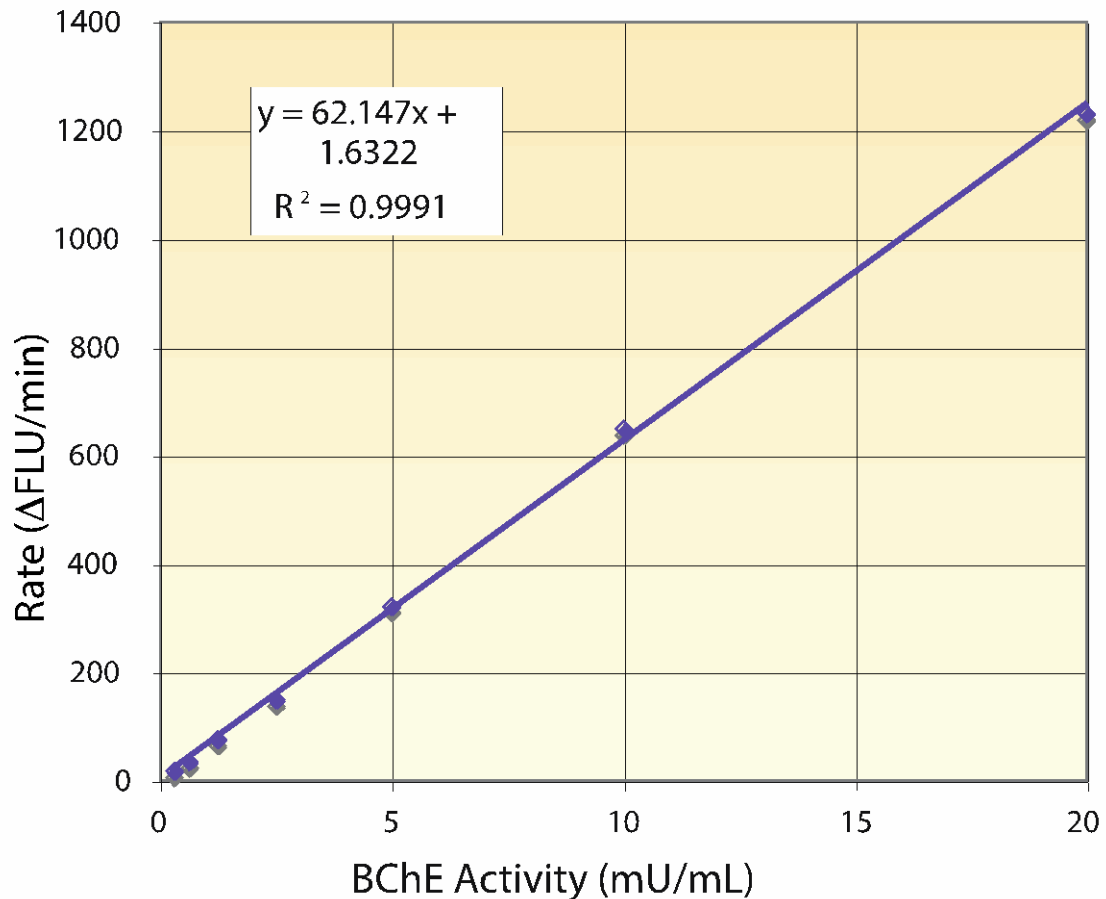


XV. Interferents:

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.

XVI. Endpoint versus Kinetic Activity:

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



XVII. References:

1. Cokugras, AN. "Butyrylcholinesterase: Structure and Physiological Importance." *Turk J Biochem* 2003; 28(2): 54-61.
2. Carmona GN, et al., "Butyrylcholinesterase accelerates cocaine metabolism: in vitro and in vivo effects in nonhuman primates and humans." *Drug Metabolism and Disposition* 1999; 28(3): 367-71.
3. Allam AR, et al., "Alzheimer's disease and Type 2 Diabetes mellitus: the cholinesterase connection?" *Lipids in Health and Disease* 2006; 5:28.
4. Greig NH, et al., " Selective Butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer beta-amyloid peptide in rodent." *Proc Natl Acad Sci USA* 2005; 102(47):17213-8.
5. Lane RM, et al., "Targeting Acetylcholinesterase and Butyrylcholinesterase in Dementia." *Int J Neuropsychopharmacol* 2006; 9(1): 101-24.