

Quantikine[®] ELISA

Human Butyrylcholinesterase/BCHE Immunoassay

Catalog Number DBCHE0

For the quantitative determination of human Butyrylcholinesterase (BCHE) concentrations in cell culture supernates, cell lysates, tissue lysates, serum, plasma, saliva, urine, and human milk.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.

614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.

19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.

24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Butyrylcholinesterase (BCHE), also known as Pseudocholinesterase or Nonspecific Cholinesterase, is a serine hydrolase that catalyzes the hydrolysis of many different choline esters. Both BCHE and the related neuronal Acetylcholinesterase (ACHE) can catalyze the hydrolysis of acetylcholine (1). BCHE can also degrade non-choline esters such as cocaine, acetyl salicylic acid, and heroin, and it exhibits aryl acylamidase activity (1-3).

In the nervous system, BCHE is expressed by glial, endothelial, and neuronal cells, (1). It assists ACHE in regulating synaptic acetylcholine levels and also participates in the regulation of cellular proliferation and neurite growth during neural development (1, 4). BCHE is also expressed in the lungs, heart, small intestine, adipose tissue, and liver (2). Liver-secreted BCHE is one of the major acetylcholine hydrolyzing enzymes found in the circulation (1).

BCHE mainly exists as a soluble, disulfide-linked, homotetrameric glycoprotein known as G4 (1, 5, 6). It can also exist as a soluble monomer and dimer, which are termed G1 and G2, respectively (1, 6). The G1 form is predominant in the developing brain, while G4 is the principal form in the mature brain (1). A cell-anchored form of G4 is composed of globular G4 bound to the Proline-rich Membrane Anchor (PRiMA) protein. Additionally, G4 BCHE can be assembled into membrane-anchored forms that contain one, two, or three tetramers attached to the plasma membrane by Collagen Q (1, 6, 7). The approximately 86 kDa subunit of human BCHE shares 80% amino acid sequence identity with the mouse and rat orthologs (5). Over 40 mutations have been identified in the BCHE gene, resulting in reduced enzymatic activity or circulating concentrations (1, 6, 8). Genetic BCHE deficiency is characterized by prolonged apnea following administration of certain anesthetic drugs such as the muscle relaxant succinylcholine (9-11).

BCHE levels have been correlated with multiple clinical conditions. Serum levels of BCHE are positively associated with the protein-synthesizing capacity of the liver and are typically reduced in patients with acute and chronic liver damage, cirrhosis, and liver metastasis (2, 12, 13). Low BCHE levels are also associated with malnutrition and pesticide toxicity (13-15). Elevated circulating BCHE is associated with low-grade systemic inflammation in hyperlipidemia, insulin resistance, obesity, metabolic syndrome, and diabetes mellitus (2, 4, 13, 16-19). In addition, BCHE is elevated in the cerebral cortex and cerebral spinal fluid of patients with Alzheimer's disease, and BCHE cortical levels are positively correlated with the number of neuritic plaques present in the cortex (1, 20, 21). Additionally, BCHE levels appear to be associated with the occurrence of stroke and the progression of several cancers including oral, renal, and pancreatic carcinoma (13, 22-25).

The Quantikine Human Butyrylcholinesterase/BCHE Immunoassay is a 4.5 hour solid phase ELISA designed to measure BCHE levels in cell culture supernates, cell lysates, tissue lysates, serum, plasma, saliva, urine, and human milk. It contains CHO cell-expressed recombinant human BCHE and antibodies raised against the recombinant protein. Results obtained for naturally occurring human BCHE showed linear curves that were parallel to the standard curves obtained using the Quantikine Human BCHE Immunoassay standards. These results indicate that this kit can be used to determine relative mass values for natural human BCHE.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human BCHE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BCHE present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human BCHE is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of BCHE bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human BCHE Microplate	894816	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human BCHE.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human BCHE Standard	894818	2 vials of recombinant human BCHE in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Discard after use. Use a new standard for each assay.
Human BCHE Conjugate	894817	21 mL of a polyclonal antibody specific for human BCHE conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with and preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. <i>Use diluted 1:5 in this assay.</i>	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples.
- Human BCHE Controls (optional; R&D Systems, Catalog # QC201).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 1 (R&D Systems, Catalog # 890713).

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- RIPA Buffer with protease inhibitors.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin[®] which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

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SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

Tissue Lysates - Tissue must be lysed prior to assay as directed in the Sample Values section.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 1000-fold dilution. A suggested 1000-fold dilution can be achieved by adding 10 μ L of sample to 240 μ L of Calibrator Diluent RD5P (diluted 1:5)*. Complete the 1000-fold dilution by adding 10 μ L of the diluted sample to 390 μ L Calibrator Diluent RD5P (diluted 1:5).

*See Reagent Preparation section.

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples.

1. Wash cells three times in cold PBS.
2. Resuspend cells a 1×10^7 cells/mL in Cell Lysis Buffer 1.
3. Incubate with gentle agitation for up to 60 minutes at room temperature.
4. Centrifuge at $8000 \times g$ for 10 minutes to remove cell debris.
5. Assay immediately or aliquot the lysis supernates and store at $\leq -70^\circ\text{C}$ until ready for use.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

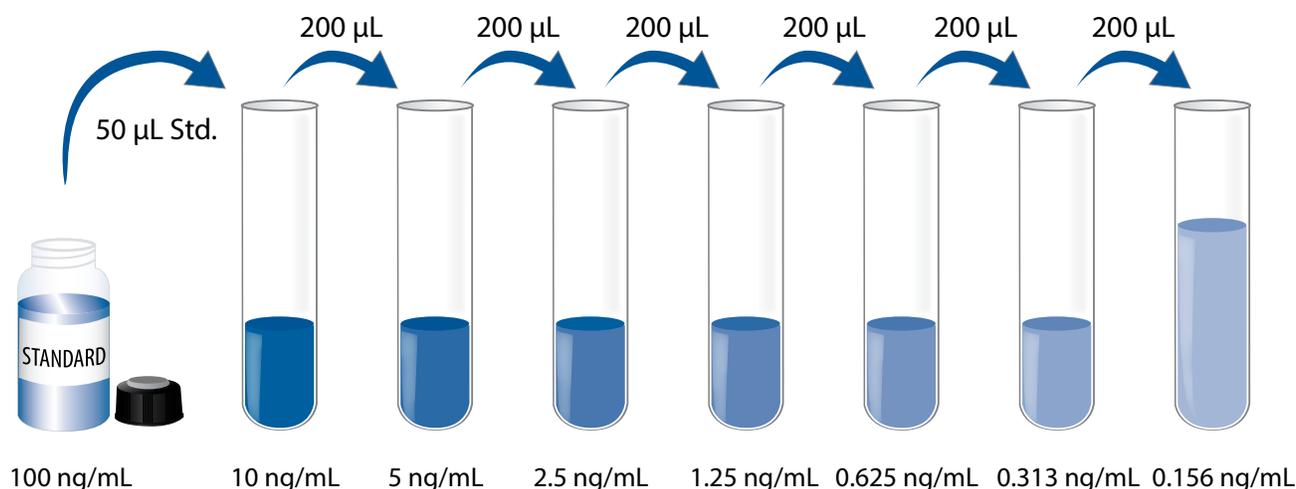
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5P Concentrate to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Human BCHE Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human BCHE Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 μL of Calibrator Diluent RD5P (diluted 1:5) into the 10 ng/mL tube. Pipette 200 μL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 μL of Assay Diluent RD1-21 to each well.
4. Add 50 μL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 μL of Human BCHE Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

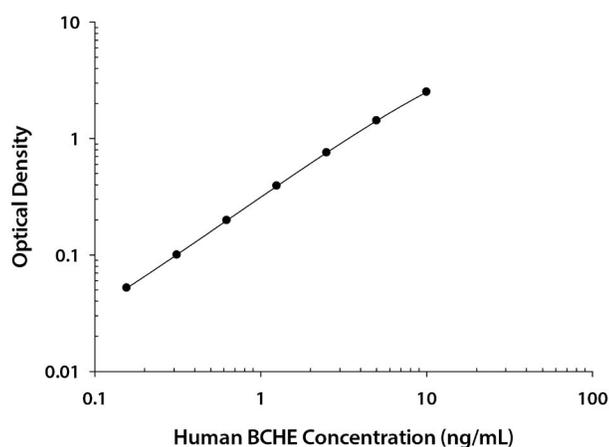
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human BCHE concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.017 0.019	0.018	—
0.156	0.070 0.070	0.070	0.052
0.313	0.117 0.118	0.118	0.100
0.625	0.215 0.216	0.216	0.198
1.25	0.402 0.416	0.409	0.391
2.5	0.771 0.772	0.772	0.754
5	1.436 1.439	1.438	1.420
10	2.509 2.523 2.537	2.523	2.505

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	1.25	2.41	5.01	1.26	2.51	5.11
Standard deviation	0.031	0.044	0.154	0.063	0.114	0.177
CV (%)	2.5	1.8	3.1	5.0	4.5	3.5

RECOVERY

The recovery of human BCHE spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	94-105%
Cell Lysis Buffer (n=2)	99	96-102
Urine (n=4)	97	93-102

SENSITIVITY

Thirty-five assays were evaluated and the minimum detectable dose (MDD) of human BCHE ranged from 0.003-0.060 ng/mL. The mean MDD was 0.011 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified CHO cell-derived recombinant human BCHE produced at R&D Systems.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human BCHE were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernate* (n=4)	Cell lysate* (n=4)	Serum* (n=4)	EDTA plasma* (n=4)
1:2	Average % of Expected	100	99	100	101
	Range (%)	97-102	98-101	98-104	100-102
1:4	Average % of Expected	99	99	101	102
	Range (%)	95-101	92-104	98-105	100-103
1:8	Average % of Expected	100	99	103	102
	Range (%)	95-104	91-101	100-107	98-104
1:16	Average % of Expected	98	96	102	104
	Range (%)	96-103	91-101	99-104	97-112

		Heparin plasma* (n=4)	Saliva (n=4)	Urine (n=4)	Human milk (n=4)
1:2	Average % of Expected	99	105	102	104
	Range (%)	97-101	103-106	99-103	98-106
1:4	Average % of Expected	102	110	105	103
	Range (%)	99-103	107-115	104-105	95-107
1:8	Average % of Expected	103	108	104	107
	Range (%)	100-107	107-109	103-105	103-111
1:16	Average % of Expected	101	98	106	111
	Range (%)	94-106	95-101	104-109	104-122

*Samples were diluted prior to assay.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human BCHE in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	4323	2292-6707	1009
EDTA plasma (n=36)	4178	2472-6131	996
Heparin plasma (n=36)	4121	2625-5882	918
Saliva (n=10)	1.95	0.157-3.98	1.23
Human milk (n=10)	3.01	1.50-5.58	1.24

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Urine (n=20)	0.427	30	ND-0.642

ND=Non-detectable

Cell Culture Supernates/Cell Lysates:

SK-Mel-28 human malignant melanoma cells were cultured in MEM supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and grown until confluent. Aliquots of the cell culture supernates were removed and assayed for human BCHE. Cells were lysed and assayed for human BCHE.

THLE-3 human liver cells were cultured in BEBM supplemented with 10% fetal bovine serum and grown until confluent. Aliquots of the cell culture supernates were removed and assayed for human BCHE. Cells were lysed and assayed for human BCHE.

Cell Line	Cell Culture Supernate (ng/mL)	Cell Lysate (ng/mg)
SK-Mel-28	3.54	44.0
THLE-3	0.753	16.6

Tissue Lysates - Brain motor cortex tissue was rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of RIPA buffer containing protease inhibitors was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. An aliquot of the lysate was removed, assayed for human BCHE, and measured 15.4 ng BCHE/mg tissue.

SPECIFICITY

This assay recognizes natural and recombinant human BCHE.

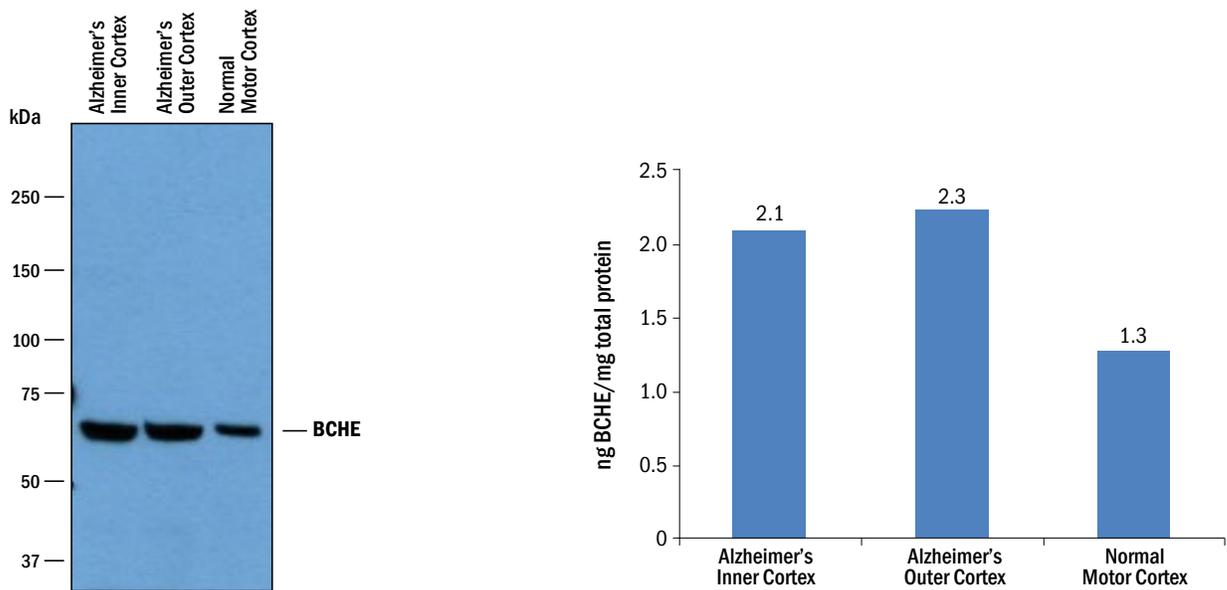
The factors listed below were prepared at 100 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human BCHE control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ACHE
IGFBP-1

Recombinant mouse:

BCHE



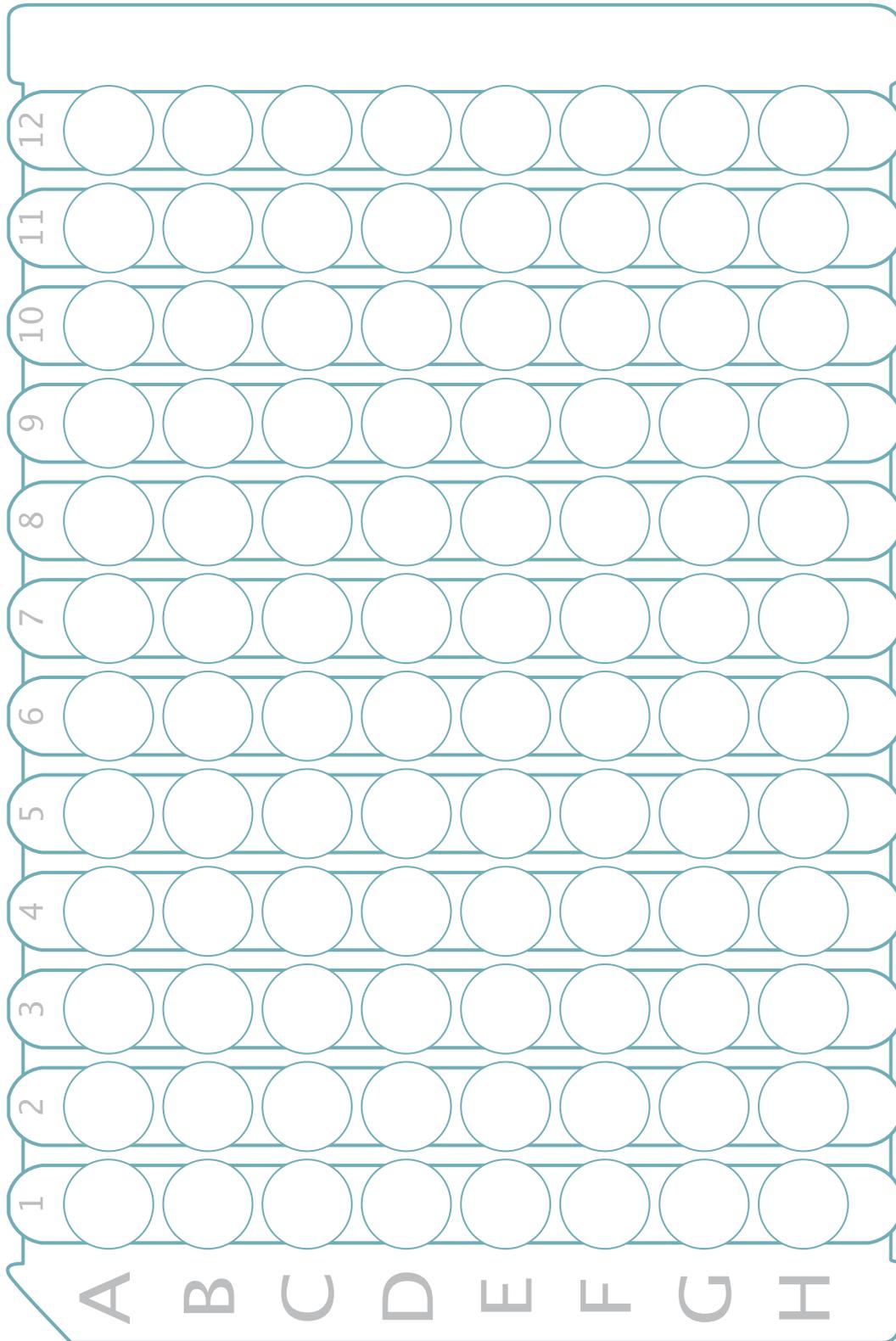
Human brain lysates from individuals with Alzheimer's disease or normal brain motor cortex (45 μ g total protein per lane) were resolved by reducing SDS-PAGE, transferred to PVDF membrane, and immunoblotted with sheep anti-human BCHE-HRP (detection antibody in the Qkit). The Western blot shows a direct correlation with ELISA sample values. BCHE has been found to be elevated in cerebral cortex in Alzheimer's disease (1, 20, 21).

REFERENCES

1. Darvesh, S. *et al.* (2003) *Nat. Rev. Neurosci.* **4**:131.
2. Lampón, N. *et al.* (2012) *Ann. Hepatol.* **11**:356.
3. George, S.T. *et al.* (1981) *Eur. J. Biochem.* **121**:177.
4. Das, U.N. (2007) *Med. Sci. Monit.* **13**:RA214.
5. Lockridge, O. *et al.* (1987) *J. Biol. Chem.* **262**:549.
6. Massoulié, J. *et al.* (1993) *Prog. Neurobiol.* **41**:31.
7. Massoulié, J. (2002) *Neurosignals* **11**:130.
8. Primo-Parmo, S.L. *et al.* (1996) *Am. J. Hum. Genet.* **58**:52.
9. Gätke, M.R. *et al.* (2005) *Anesthesiology* **102**:503.
10. Levano, S. *et al.* (2005) *Anesthesiology* **102**:531.
11. Garcia, D.F. *et al.* (2011) *Genet. Mol. Biol.* **34**:40.
12. Pohanka, M. *et al.* (2013) *Bratisl. Lek Listy* **114**:726.
13. Santarpia, L. *et al.* (2013) *J. Cachexia Sarcopenia Muscle* **4**:31.
14. Rastogi, S.K. *et al.* (2008) *Indian J. Occup. Environ. Med.* **12**:29.
15. Wafa, T. *et al.* (2013) *J. Environ. Sci. Health B.* **48**:1058.
16. Abbott, C.A. *et al.* (1993) *Clin. Sci.* **85**:77.
17. Rao, A.A. *et al.* (2007) *Med. Hypotheses* **69**:1272.
18. Sridhar, G.R. *et al.* (2010) *Med. Hypotheses* **75**:648.
19. Sato, K.K. *et al.* (2014) *Clin. Endocrinol.* **80**:362.
20. Sáez-Valero, J. *et al.* (2003) *J. Neurosci. Res.* **72**:520.
21. Flirski, M. and T. Sobow (2005) *Curr. Alzheimer Res.* **2**:47.
22. Mitsunaga, S. *et al.* (2008) *Pancreas* **36**:241.
23. Ben Assayag, E. *et al.* (2010) *Mol. Med.* **16**:278.
24. Prabhu, K. *et al.* (2011) *Australas. Med. J.* **4**:374.
25. Koie, T. *et al.* (2014) *Scientific World Journal* **2014**:948305.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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