VALIDATION DATA

Butyrylcholinesterase Activity Assay Kit

Item No. 502895

The Butyrylcholinesterase Activity Assay Kit was assessed using a variety of tests and procedures to validate its performance for the quantitative measurement of butyrylcholinesterase (BChE) activity in biological matrices commonly used with this kit. The validation experiments were designed to evaluate assay sensitivity, working range, precision, accuracy, and matrix compatibility under the conditions described in the kit booklet.

The data presented in this document describe the performance characteristics of the assay and are intended to assist users in experimental planning and interpretation of results.

Parameter	Performance Metric
Detection method	Colorimetric (410 nm)
Assay duration	45 minutes
Limit of detection (LOD)	0.5 mU/ml
Lower limit of quantification (LLOQ)	1 mU/ml
Sample volume	20 µl
Species compatibility	Multiple species*
Validated species	Human, mouse, rat, pig
Validated matrices	Serum, plasma, tissue homogenates, cell lysates

**NOTE: This assay is expected to be compatible with samples from species expressing BChE; however, performance has not been validated across all species.*

Assay Range and Sensitivity

The assay demonstrated a linear response over an activity range up to 0.17 ΔA_{410}/min using the positive control (purified, recombinant human BChE) under the conditions described in the kit protocol.

The limit of detection (LOD) represents the lowest activity that can be reliably distinguished from the background signal. The LOD for the assay is 0.5 mU/ml BChE.

The lower limit of quantification (LLOQ) represents the lowest activity that can be quantified with acceptable precision. The LLOQ for the assay is 1 mU/ml BChE.

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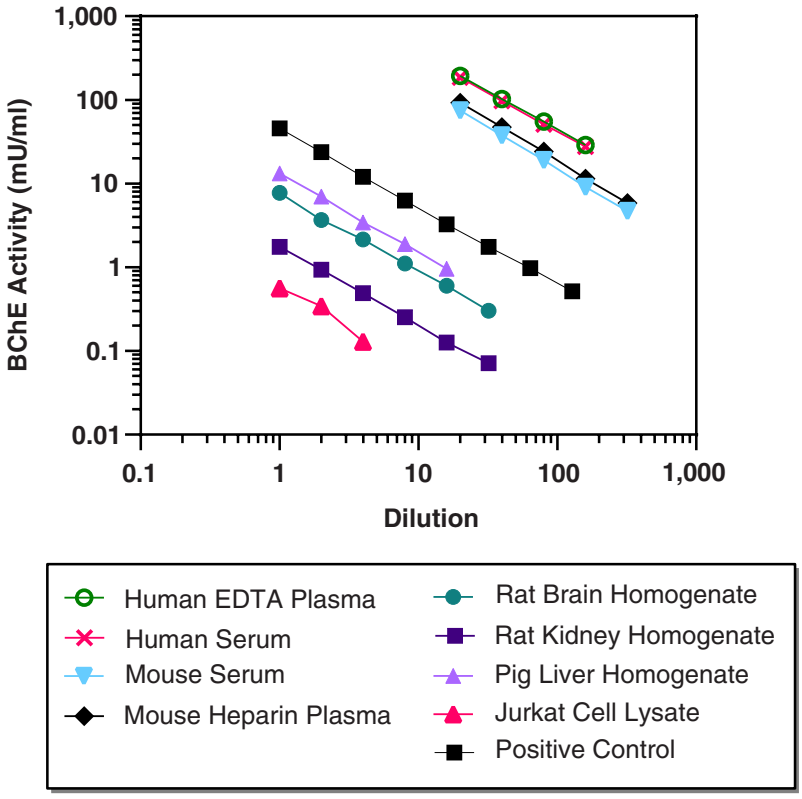
Dilutional Linearity and Parallelism

Dilutional linearity experiments demonstrate whether measured enzymatic activities in samples proportionally decrease with dilution. The parallelism of the sample dilution curves to the positive control dilution curve indicate that assay performance is not differentially affected by sample matrix properties.



Linearity of various matrices. Samples were serially diluted with assay buffer and analyzed using the Butyrylcholinesterase Activity Assay Kit. Measured activities were plotted as a function of sample dilution.

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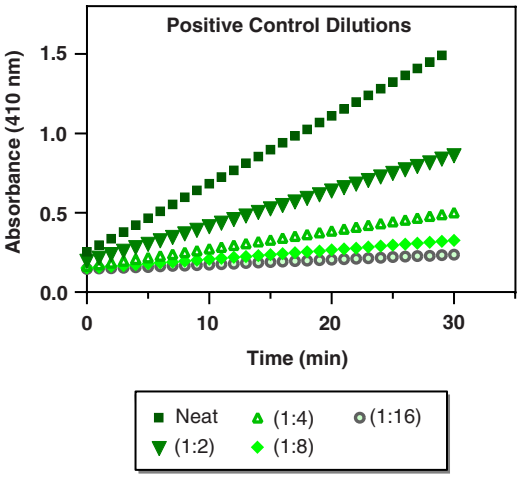
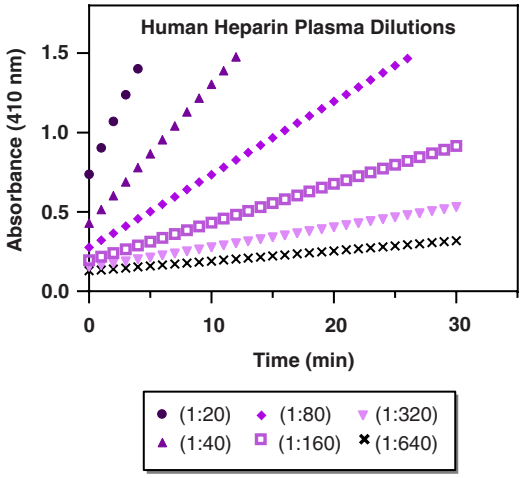
Assay Range

To determine the assay range, the positive control and human heparin plasma were serially diluted and tested in the assay. The dilution series demonstrated a linear response supporting accurate quantification between 1-178 mU/ml BChE activity in validated matrices.

A



B



C

BChE Positive Control		
Dilution	ΔA_{410}/min	BChE Activity, mU/ml
--	0.0428	46
1:2	0.0223	24
1:4	0.0113	12
1:8	0.00583	6.2
1:16	0.00304	3.3
1:32	0.00163	1.7
1:64	0.00091	1.0
1:128	0.00048	0.5
Human Heparin Plasma		
Dilution	ΔA_{410}/min	BChE Activity, mU/ml
1:20	0.166	178
1:40	0.0874	94
1:80	0.0459	49
1:160	0.0242	26
1:320	0.0126	13
1:640	0.0064	6.9

Assay range determination. (A) Positive control and (B) human heparin plasma were serially diluted with assay buffer and analyzed using the Butyrylcholinesterase Activity Assay Kit. (C) Absorbance at 410 nm was measured over time and used to calculate the BChE activity.

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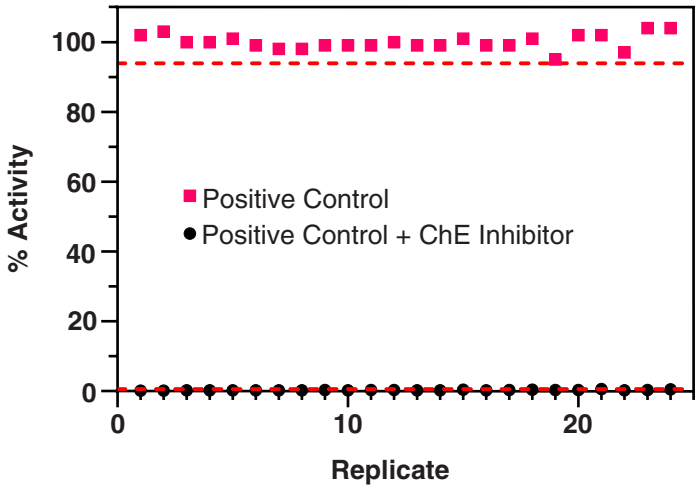
Z' Factor

Z' factor is a term used to describe the robustness of an assay, which is calculated using the equation below[1]:

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

Where σ: Standard deviation
μ: Mean
c+: Positive control
c-: Negative control

The theoretical upper limit of the Z' factor is 1.0. A robust assay has a Z' factor >0.5. The Z' factor for this assay kit was determined to be 0.94.



Typical performance data. Data are shown from 24 replicates each for the positive control and positive control with a cholinesterase (ChE) inhibitor prepared as described in the kit booklet. The calculated Z' factor for this experiment was 0.94. The red lines correspond to three standard deviations from the mean for each control value.

Precision

The coefficient of variation (CV) is a measurement of consistency between sample replicates. Precision was evaluated both within a single assay run (intra-assay) and across multiple runs (inter-assay) on different days, using rat brain tissue and human EDTA plasma at representative concentrations within the assay range.

Sample	*%CV Intra-Assay Precision	*%CV Inter-Assay Precision
Rat brain homogenate	3.7	6.5
Human EDTA plasma	5.0	6.8

Intra- and inter-assay precision. A series of 24 replicate measurements were performed on the same day to determine intra-assay CVs. A series of six measurements were performed on different days under the same experimental conditions to determine inter-assay CVs.
*%CV represents the variation in calculated concentrations between replicate measurements.

Reference

1. Zhang, J.H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assay. *J. Biomol. Screen.* **4(2)**, 67-73 (1999).

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