Butyryl Cholinesterase Microplate Assay Kit User Manual

Catalog # CAK1291

(Version 1.1A)

Detection and Quantification ofButyryl Cholinesterase (BChE)Activityin Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Butyryl Cholinesterase (BChE, EC 3.1.1.8) is sometimes referred to as pseudocholinesterase but it preferentially uses butyrylcholine and benzoylcholine as substrates. Butyrylcholinesterase is distinct from acetylcholinesterase and is found in mammalian blood plasma, liver, pancreas, intestinal mucosa and the white matter of the central nervous system.Butyrylcholinesterase has a molecular weight of 440,000. It is a glycoprotein which has an optimum pH of 6.0-8.0. Assay of butyrylcholinesterase activity is of diagnostic value in various liver diseases, malignancies, and pulmonary tuberculosis. The enzyme can also be used for the assay of organophosphorous compounds such as pesticides.

Butyryl CholinesteraseMicroplate Assay Kitprovides a simple and sensitive method for monitoring Butyryl Cholinesterase activity in various samples.The enzyme catalysed reaction productsp-nitrophenol can be measured at a colorimetric readout at 412 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powderx 1	4 °C, keep in dark
Dye Reagent	Powderx 1	4 °C, keep in dark
Standard	Powderx 1	4 °C
Positive Control	Powderx 1	-20 °C
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Note:

Substrate: add 1 ml distilled water to dissolve before use; store at 4 °C for 1 monthafter reconstitution.

Dye Reagent: add 1 ml ethanol to dissolve before use; store at 4 °C for 1 weekafter reconstitution.

Standard:add 1 mldistilled water to dissolve before use; then 250µl into 750µldistilled water, the concentration will be 5mmol/L; store at -20°C for 1 monthafter reconstitution.

Positive Control: add 1 ml Assay Bufferto dissolve before use; store at -80 °C for a monthafter reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 412 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	170µl	170µl	170µl	170µl	170µl
Substrate	10 µl	10 µl	--	--	10 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	10 µl	20 µl	--
Standard	--	--	10 µl	--	--
Positive Control	--	--	--	--	10 µl
Dye Reagent	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, wait for 2 minutes, record absorbance measured at 412 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Butyryl Cholinesterase activity is defined asthe enzyme hydrolyze 1μmol of butyrylthiocholine iodide per minute at 25°C and pH 7.4.

1. According to the protein concentration of sample

$$BChE\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= 2.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$BChE\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 2.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

$$BChE\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 2.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$BChE\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 2.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 5 mmol/L = 5μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of the standard, 0.01 ml;

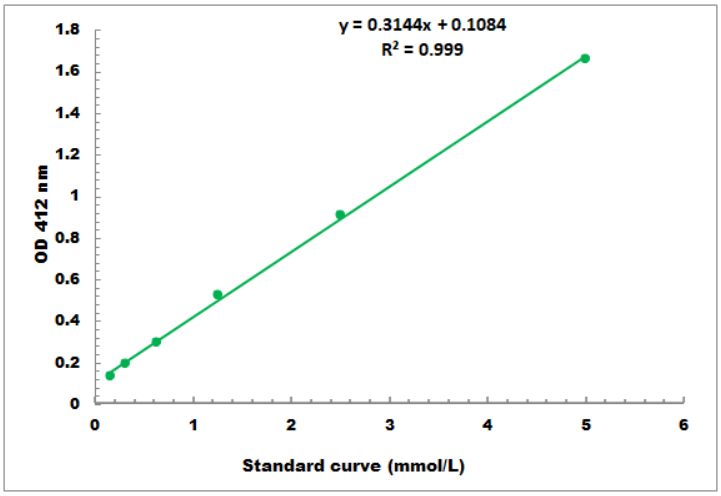
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

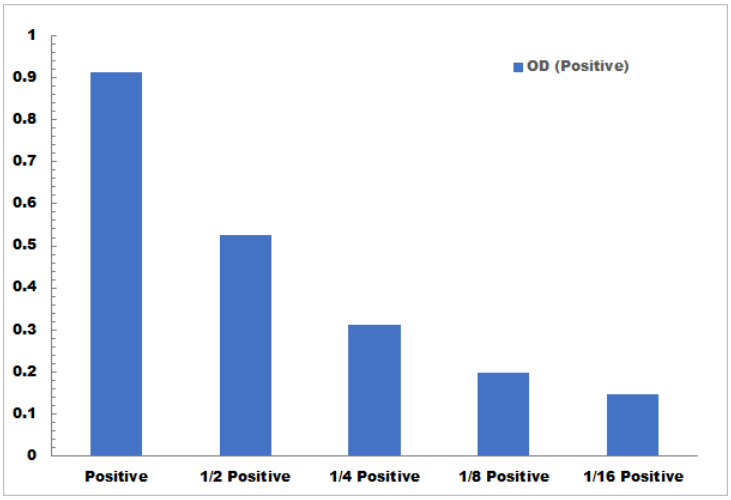
T: the reaction time, 2 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 5 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES