

Butyryl Cholinesterase Microplate Assay Kit

User Manual

Catalog # CAK1291

(Version 1.1A)

Detection and Quantification of Butyryl Cholinesterase (BChE) Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

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



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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 Cholinesterase Microplate Assay Kit provides a simple and sensitive method for monitoring Butyryl Cholinesterase activity in various samples. The enzyme catalysed reaction products p-nitrophenol can be measured at a colorimetric readout at 412 nm.



II. KIT COMPONENTS

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

Note:

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

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

Standard: add 1 ml distilled water to dissolve before use; then 250 μ l into 750 μ l distilled water, the concentration will be 5 mmol/L; store at -20 °C for 1 month after reconstitution.

Positive Control: add 1 ml Assay Buffer to dissolve before use; store at -80 °C for a month after 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 ml Assay buffer for 5×10^6 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

For liquid samples
Detect directly.



V. ASSAY PROCEDURE

Reagent	Sample	Control	Standard	Blank	Positive		
					Control		
Reaction Buffer	170 µl	170 µl	170 µl	170 µl	170 µl		
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.							

Add following reagents into the microplate:

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.

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VI. CALCULATION

Unit Definition: One unit of Butyryl Cholinesterase activity is defined as the enzyme hydrolyze 1 μ mol 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} × V_{Standard}) × (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} × V_{Sample}) / T

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

2. According to the weight of sample

$$\begin{split} & \text{BChE} \left(\text{U/g} \right) = \left(\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}} \right) \times \left(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} \right) / \left(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}} \right) / \left(\text{V}_{\text{Sample}} \times \text{W} / \text{V}_{\text{Assay}} \right) / \text{T} \end{split}$$

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

3. According to the quantity of cells or bacteria

BChE (U/10⁴) = (C_{Standard} × V_{Standard}) × (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 = 5 μ mol/ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

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 to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES