Acetylcholinesterase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1068

(Version 2.2E)

Detection and Quantification of Acetylcholinesterase(AChE)Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

Acetylcholinesterases (AChEs) are enzymes that hydrolyze the neurotransmitter acetylcholine (ACh) to and choline. AChE is located at the synaptic cleft and functions to terminate synaptic transmission by catalyzing the breakdown of ACh allowing cholinergic neurons to return to a resting state after activation. Changes in AChE activity may result from exposure to certain insecticides, which act as cholinesterase inhibitors. Inhibitors of AChE are also used to treat certain conditions such as dementia.

Acetylcholinesterase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring AChE levels in a variety of samples such as blood, serum, and plasma. In this assay, thiocholine produced by AChE, reacts with DTNB to form an colorimetric (412 nm) product (TNB), proportional to the AChE activity present.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 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
Technical Manual	1 Manual	

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
9. Ethanol

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 20 mmol/L of standardstock solution, store at -20°C for 1 monthafter reconstitution.Then dilute to 5mmol/Lstandard top solutionby adding 250 µlstock solution into 750 µldistilled water**.** Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078mmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use; store at 4 °C for 1 monthafter reconstitution.

Dye Reagent: Briefly centrifuge prior to opening. Add 1 ml ethanol to dissolve before use; keep in dark and store at 4 °C for 1 weekafter reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Bufferto dissolve before use; store at -80 °C for a monthafter reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 4000g 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 4000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Warm Reaction Bufferto room temperature before use.

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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of AChE activity is defined as the enzyme hydrolyze 1μmol of Acetylthiocholine iodide per minute at 25°C and pH 7.4.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

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

$V_{Standard}$: the volume of standard in assay procedure, mL

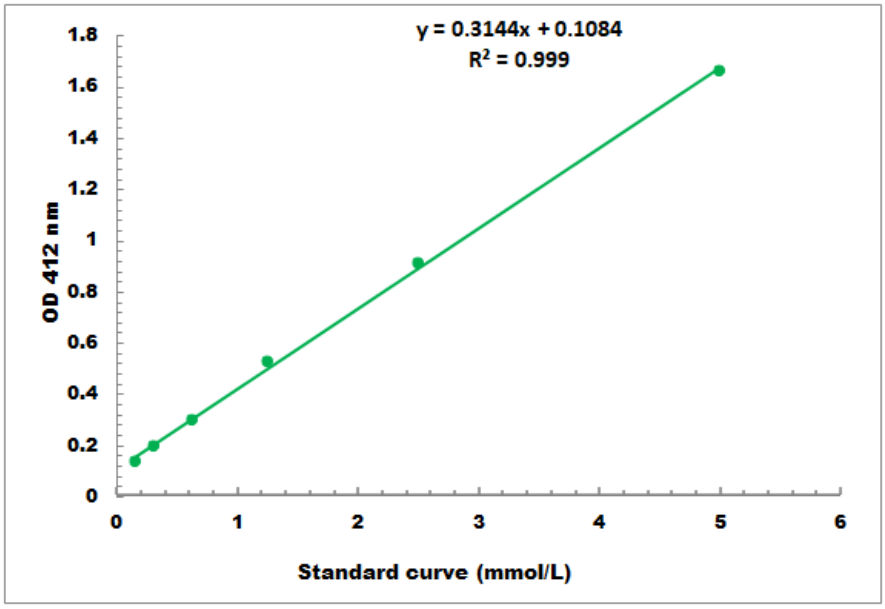
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

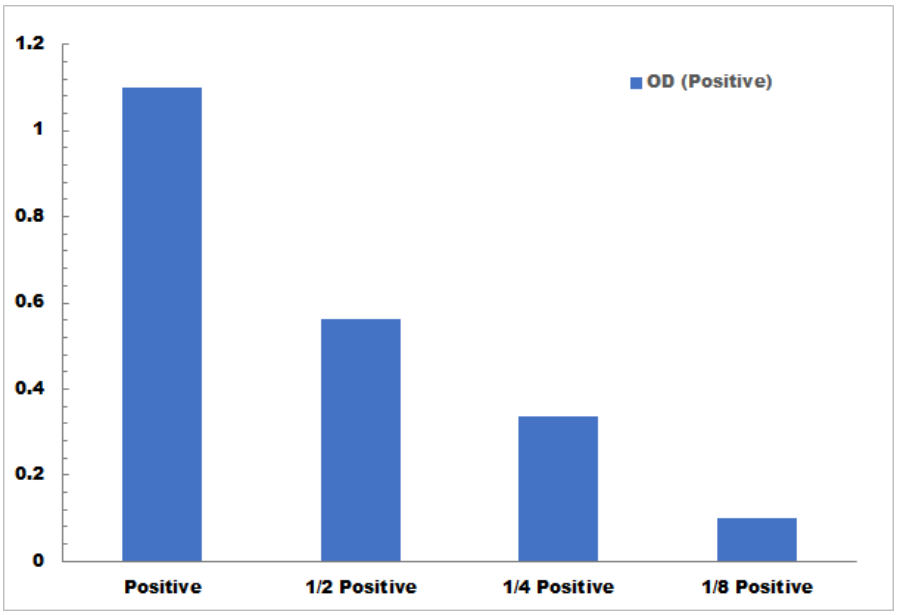
T: the reaction time, minute

VIII. 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