Acetylcholinesterase Microplate Assay Kit User Manual

Catalog # ASK1068

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

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

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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.

The Acetylcholinesterase Activity 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.

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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
Dye Reagent	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml Reaction Buffer to dissolve before use.

Dye Reagent: add 1 ml ethanol to dissolve before use.

Positive Control: add 1 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 412 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
9. Ethanol

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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%, sonication 3s, intervation 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

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

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V. ASSAY PROCEDURE

Warm Reaction Buffer to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Blank	Positive Control
Reaction Buffer	160 µl	160 µl	160 µl
Substrate	10 µl	10 µl	10 µl
Dye Reagent	10 µl	10 µl	10 µl
Sample	20 µl	--	--
Distilled water	--	20 µl	--
Positive Control	--	--	20 µl
Mix, measured at 412 nm and record the absorbance of 20th second and 200th second.			

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VI. CALCULATION

Unit Definition: One unit of AchE activity is defined as the enzyme generates 1 μmol of TNB per minute.

1. According to the protein concentration of sample

$$AchE\ (U/mg) = [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.408 \times [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})] / C_{Protein}$$

2. According to the weight of sample

$$AchE\ (U/g) = [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})] / (\varepsilon \times d) \times V_{Total} / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.408 \times [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})] / W$$

3. According to the quantity of cells or bacteria

$$AchE\ (U/10^4) = [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})] / (\varepsilon \times d) \times V_{Total} / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.408 \times [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})] / N$$

4. According to the volume of serum or plasma

$$AchE\ (U/ml) = [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})] / (\varepsilon \times d) \times V_{Total} / V_{Sample} / T$$

$$= 0.408 \times [(OD_{Sample(200S)} - OD_{Sample(20S)}) - (OD_{Blank(200S)} - OD_{Blank\ (20S)})]$$

ε: molar extinction coefficient, 13.6×10^3 L/mol/cm = 13.6 ml/μmol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

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

W: the weight of sample, g;

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

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.02 ml;

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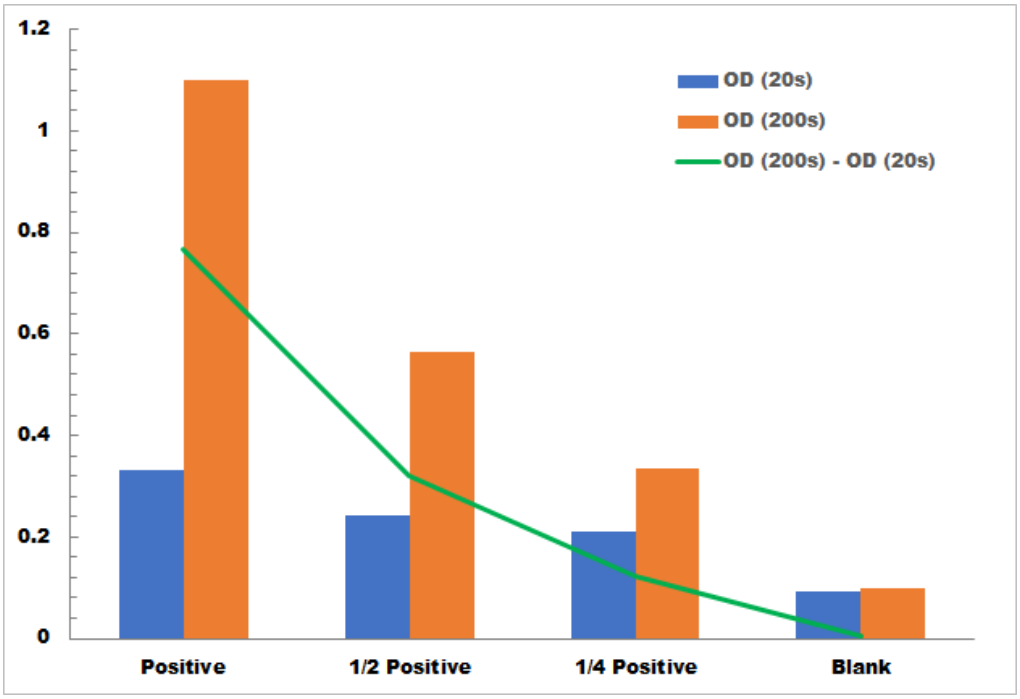
V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 3 minutes.

VII. TYPICAL DATA



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

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