Cholesterol Esterase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1294

(Version 1.1A)

Detection and Quantification of Cholesterol Esterase (CE) Activity in Serum, Plasma, Tissue extracts, Cell lysate and Other biological fluids and Other biological fluids Samples.

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

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I. INTRODUCTION

Cholesterol Esterase (CE) catalyzes the hydrolysis of cholesteryl esters into free cholesterol and fatty acids, serving as a key enzyme in cholesterol absorption, transport, and storage. By modulating intracellular free cholesterol levels, this enzyme participates in essential physiological processes including lipid homeostasis, cell membrane biosynthesis, and steroid hormone production.

Cholesterol Esterase Activity Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of Cholesterol Esterase (CE) activity in a variety of samples. Cholesterol Esterase converts cholesterol ester to cholesterol and fatty acid. H2O2 is generated through a linked enzymatic reaction, which reacts with dye reagent to form a colored product. The color intensity increase at 550nm is proportional to the Cholesterol Esterase activity in the sample.

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 I	Powder x 1	4 °C, keep in dark
Substrate I Diluent	1 ml x 1	4 °C
Substrate II	Powder x 1	4 °C, keep in dark
Enzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	4 °C, keep in dark
Positive control	Powder x 1	-20 °C, keep in dark
Standard Diluent	12 ml x 1	4 °C
Standard	Powder x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water and heated Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. 80°C water bath

IV. REAGENT PREPARATION

Substrate Working Solution: Add 1.0 mL of Substrate I Diluent to the bottle of Substrate I, and dissolve completely by heating to 80°C. Slowly add 18.2 mL of 80°C preheated distilled water along the wall of the beaker with stirring to maintain clarity, then cool under running tap water and shake to mix. Then add the cooled all Substrate I solution into the bottle of Substrate II, and dissolve well by vertexing. Keep in dark and store at 4 °C for 5 days or -20 °C for 2 months.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Keep in dark and store at 4 °C for 1 week or -20 °C for 3-4 months.

Dye Reagent: Add 10 ml Reaction Buffer to dissolve before use. Store at 4 °C for 2-3 weeks or -20 °C for 2 months.

Positive Control: Briefly centrifuge prior to opening. Add 0.5 ml Reaction Buffer to dissolve before use. Keep in dark and store at 4 °C for 5 days or -20 °C for 3-4 months.

Standard: Briefly centrifuge prior to opening. Add 0.5 ml Standard Diluent to dissolve to generate 10 mmol/L of standard top solution. Store at 4 °C for 1 week or -20°C for 1-2months after reconstitution. Perform 2-fold serial dilutions of the top standard solution using Assay Buffer to make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/ 0.156 mmol/L.

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

V. SAMPLE PREPARATION

1. For cell 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 (the quantity should be adjusted according to the actual situation of the sample), sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 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 (the quantity should be adjusted according to the actual situation of the sample), homogenize with 1 ml Assay Buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	70 µl	70 µl	70 µl	70 µl	70 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	10 µl	20 µl
Positive Control	--	--	10 µl		
Substrate Working Solution	10 µl	10 µl	10 µl	--	--
Standard	--			10 µl	--
Enzyme	10 µl	10 µl	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, 37 °C wait for 10 minutes, measure at 550 nm and record the absorbance.					

Note:

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

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**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 Cholesterol Esterase is defined as the enzyme catalyzes the hydrolysis of 1 μmol of cholesteryl ester per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the quantity of cells

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

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

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

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

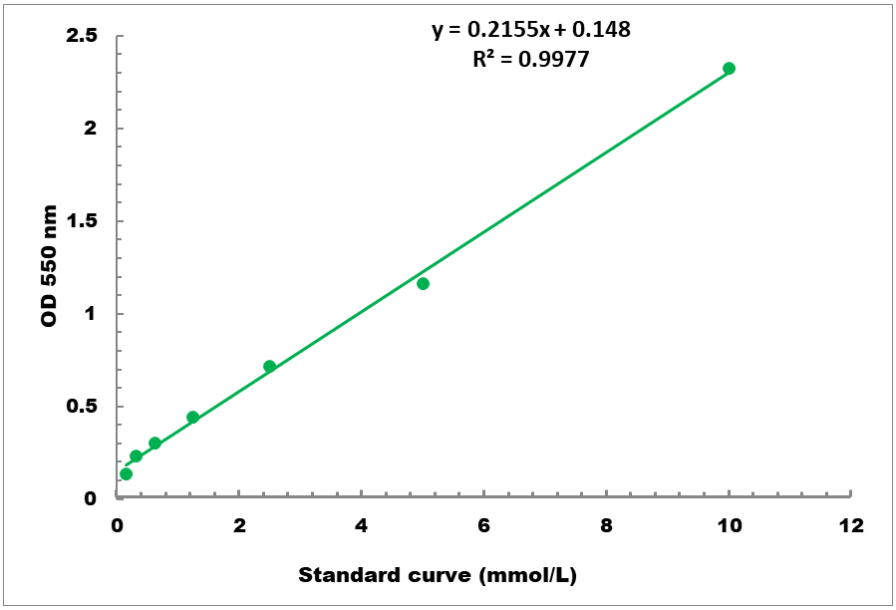
V_{Sample}: the volume of sample in assay procedure, mL

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

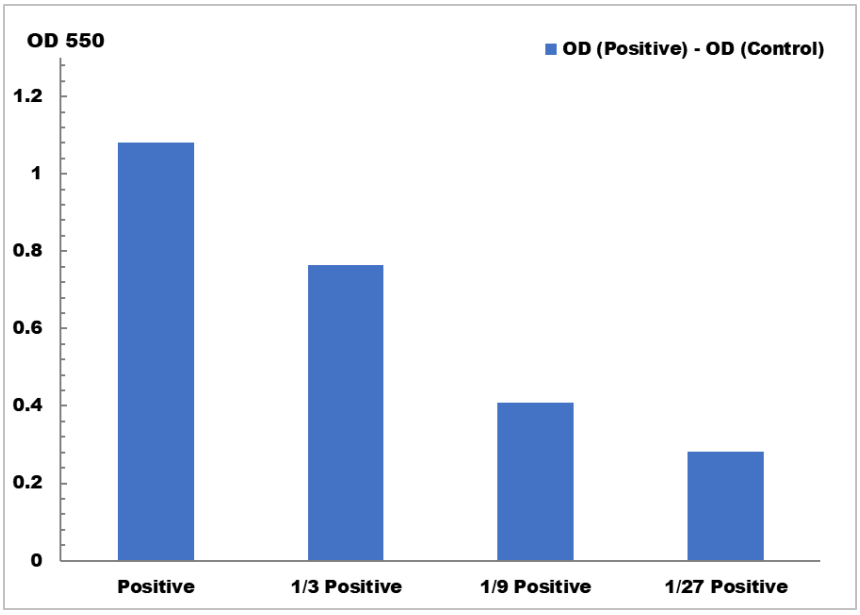
T: the reaction time, minute

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 - 10 mmol/L



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