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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Cholesterol esterase (CE)

Cat no. LDG0028RG

Product Overview

Specification

Appearance	White amorphous powder, lyophilized
Activity	70 U/mg or more

Properties

Stability	Stable at −20℃ for at least one year
Molecular weight	52 kDa
Isoelectric point	5.5

Assay

1. Principle

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

Cholesterol $\xrightarrow{\text{Cholesterol oxidase}}$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + Phenol $\xrightarrow{\text{Peroxidase}}$ Quioneimine dye + $4H_2O$

The appearance of quinoneimine dye formed when coupled with 4-aminoantipyrine, and phenol is measured at 505 nm by spectrophotometry.

2. Definition

One unit causes the formation of one micromole of hydrogen peroxide (half a micromole of quinoneimine dye) per minute under the detailed conditions below.

3. Reagents

A. Enzyme diluent	Prepare the buffer containing 20 mM K_2HPO4 and adjust the pH value to 7.5 with KOH. Then, add 0.019 g of $MgCl_2$, 0.0186 g of EDTA-2Na, 0.2 g of BSA, and 0.1 g of sodium azide to the buffer, and adjust the pH value to exactly 7.0 with KOH. Fill up the solution to 100 mL with distilled water.
B. Reagent 1	Add 1.4 g of KH_2PO_4 to 80 mL of distilled water. Then, add 0.0961 g of 4-Aminoantipyrine (4-AA), 1500 U of Peroxidase (POD), 3000 U of Cholesterol oxidase, and 0.1 g of sodium azide. Adjust the pH value to exactly 7.0 with KOH. Fill up the solution to 100 mL with distilled water.

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C. Reagent 2	Add 1.4 g of KH_2PO_4 to 80 mL of distilled water. Then, add 0.6 g of phenol, and 0.2 g of sodium azide. Use KOH to adjust the pH value to exactly 7.0. Fill up the solution to 100 mL with distilled water.
D. Reagent 3	Add 1 g of Triton X-100 to 80 mL of distilled water, and heat to 70℃. Then, add 39 mg of cholesterol eater (dissolved in 2 mL isopropanol), and stir to dissolve. Stop heating until the solution becomes clear in 3-5 minutes. Add 0.6 g of sodium tauroglycocholate as the solution cold down to 60℃. Fill up the solution to 100 mL with distilled water.

4. Procedure

(1) Prepare the following working solution (for 10 tests) in a brownish bottle.

Working solution

Reagent 1 (B)	3 mL
Reagent 2 (C)	4.5 mL
Reagent 3 (D)	4.5 mL
Total	**12 mL**

(2) Pipette 1.2 mL of working solution into a cuvette (d = 1.0 cm) and equilibrate at 37℃ for approximately 3 minutes. Add 0.04 mL of sample solution, mix and keep at 37℃ for another 2 minutes.

(3) Record the increase in optical density at 505 nm against water for 3 to 4 minutes in a spectrophotometer at 37℃ and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test). At the same time, measure the blank rate (ΔOD blank) using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.

*Dissolve the enzyme preparation in ice-cold enzyme diluent **(Reagent A)**, dilute to **0.1–0.3 U/mL** with the same buffer, immediately before the assay.

(4) Activity can be calculated by using the following formula:

Volume activity (U/mL) =

$$\frac{\Delta OD/\text{ min } (\Delta OD \text{ test} - \Delta OD \text{ blank}) \times Vt \times df}{13.78 \times 1/2 \times 1.0 \times Vs}$$

$$= \Delta OD/\text{min} \times 4.499 \times df$$

Weight activity (U/mg) = (U/mL)×1/C

Vt: Total volume (mL)

Vs: Sample volume (mL)

13.78: Millimolar extinction coefficient of quinoneimine dye under the assay conditions (cm^2/ micromole)

1/2: Factor based on the fact that one mole of H_2O_2 produces half a mole of quinoneimine dye.

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration in dissolution (mg/ mL)

The effect of different conditions on Cholesterol esterase

A.

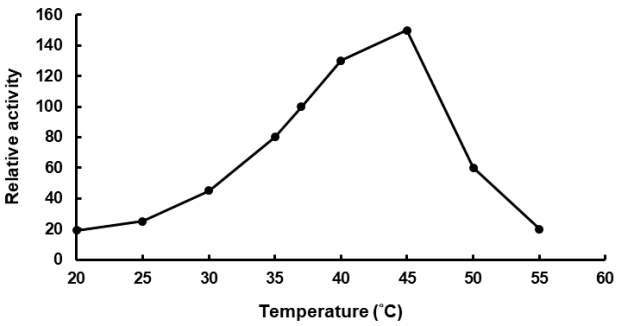

Figure A. Temperature activity of Cholesterol esterase. The enzyme reactions in 0.1 M Sodium acetate buffer, pH 5.5, were carried out under different temperatures.

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B.

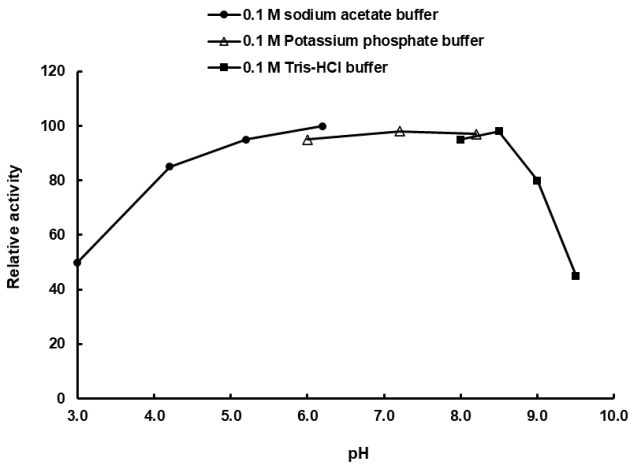

Figure B. pH activity of Cholesterol esterase. The buffer conditions with various pH values were used in the reaction.

C.

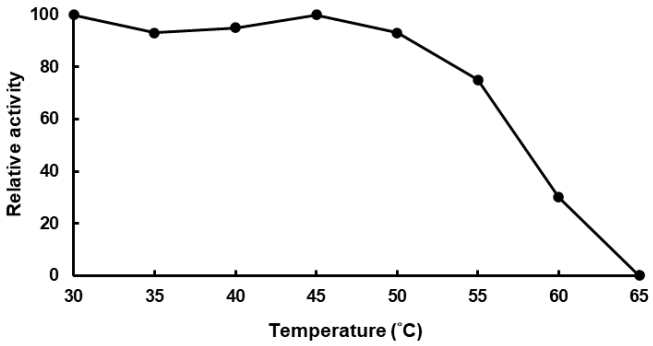

Figure C. Thermal stability of Cholesterol esterase. The enzyme powder was reconstituted by double-distilled water and treated with different temperatures for 15 minutes.

D.

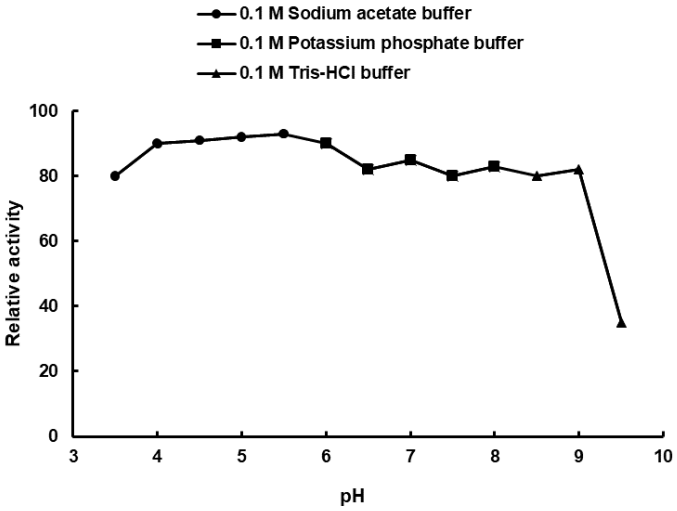

Figure D. pH stability of Cholesterol esterase. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer conditions for 24 hours.

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