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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Cholesterol oxidase (CO)

Cat no. LDG0025RG

Product Overview

Specification

Appearance	Yellow amorphous powder, lyophilized
Activity	40 U/mg or more

Properties

Stability	Stable at −20℃ for at least one year
Molecular weight	58.9 kDa
Isoelectric point	8.52

Assay

1. Assay principle

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrin + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + $4H_2O$

The appearance of quinoneimine dye formed when coupled with 4-aminoantipyrine, and phenol is measured at 500 nm by spectrophotometry.

2. Unit definition

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

3. Reagents

A. K-phosphate buffer, pH 7.0	0.1 M
B. Cholesterol solution	Mix 500 mg of cholesterol and 5 mL of Triton X-100 on a hot plate or in a water bath with stirring until cholesterol dissolves. Add 90 mL of distilled water to the mixture by slowly pouring. Stir and allow to boil for 30 to 60 seconds. The solution will become cloudy. Cool down with gentle agitation until the solution turns clear. Add 4 g of sodium azide and dissolve it to make a final concentration of 4%. Fill up the solution to 100 mL with distilled water.
C. 4-AA solution	1.76% (1.76 g 4-aminoantipyrine in 100 mL of H_2O)
D. Phenol solution	6.0% (6.0 g phenol in 100 mL of H_2O)
E. POD solution	Horseradish peroxidase:15,000 purpurogallin units in 100 mL of buffer (A)
F. Enzyme diluent	20 mM K-phosphate buffer, pH 7.0 containing 0.2% BSA

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4. Procedure

(1) Prepare the following **working solution** immediately before use and equilibrate in a brownish bottle at 37℃ for approximately 3 minutes (for 4 reactions).

Working solution

0.1 M K-phosphate buffer, pH 7.0 (Reagent A)	5.1 mL
Cholesterol solution (Reagent B)	0.4 mL
4- AA solution (Reagent C)	0.1 mL
POD solution (Reagent E)	0.2 mL
Total	**5.8 mL**

(2) Pipette 1.45 mL of working solution into a tube.

(3) Add 0.05 mL of Phenol solution (Reagent D), mix by gentle inversion, and keep at 37℃ for another 2 minutes.

(4) Add 0.05 mL of the enzyme solution and mix by gentle inversion.

Concentration in a reaction	
K-phosphate buffer	87 mM
Cholesterol	0.89 mM
4-AA	1.4 mM
Phenol	21 mM
Triton X-100	0.34%
Sodium cholate	64 mM
BSA	33 μg/mL
POD	5 U/mL

(5) Pipette 1 mL of the mixture into a cuvette (d=1.0 cm).

(6) Record the increase in optical density at 500 nm against water for 1 to 5 minutes in a spectrophotometer at room temperature 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) by using the same method as the test except that the enzyme diluent is added instead of enzyme solution.

* Dilute the enzyme in ice-cold enzyme diluent (**Reagent E**) to **0.1–0.3 U/mL** and store on ice.

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

Volume activity (U/mL) =

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

$= \Delta OD/min \times 4.499 \times df$

Weight activity (U/mg) = (U/mL) × 1/C

Vt: Total volume (1.55 mL)

Vs: Sample volume (0.05 mL)

13.78: Millimolar extinction coefficient of NADH at 340 nm (cm²/micromole)

C: Enzyme concentration in dissolution (mg/mL)

The effect of different conditions on Cholesterol oxidase

A.

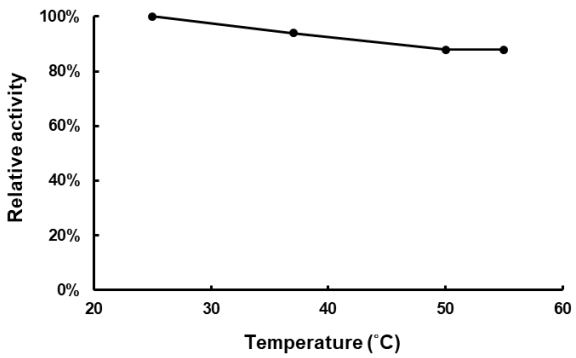

Figure A. Temperature activity of Cholesterol oxidase. The enzyme reactions in 0.1 M Potassium phosphate buffer, pH 7.0, were carried out under different temperatures.

B.

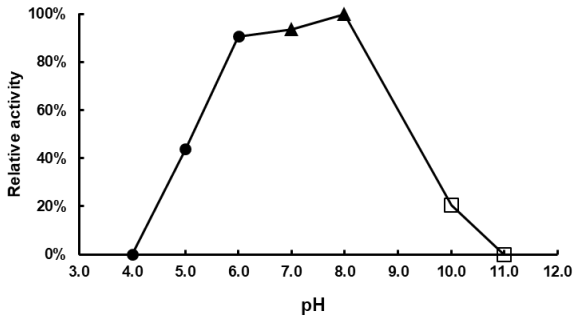

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Figure B. pH activity of Cholesterol oxidase. The buffer conditions with various pH values were used in the reaction at 37°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

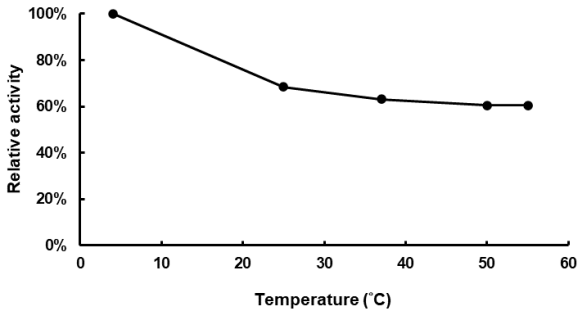

Figure C. Thermal stability of Cholesterol oxidase. The enzyme powder was reconstituted by double-distilled water and treated with different temperatures for 15 minutes. Final concentration: 4.3 U/mL

D.

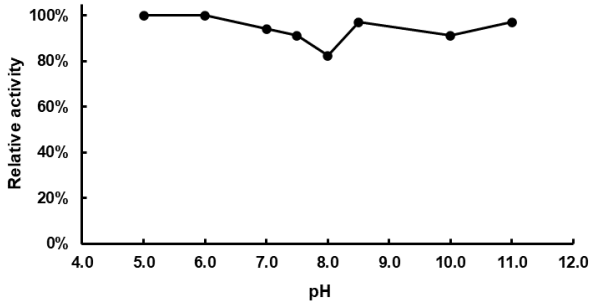

Figure D. pH stability of Cholesterol oxidase. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 20 hours. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

Disclaimer

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