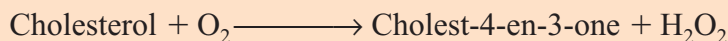


# Cholesterol Oxidase (CHO-CE)

from recombinant *E. coli*

Cholesterol : oxygen oxidoreductase, EC 1.1.3.6



## SPECIFICATION

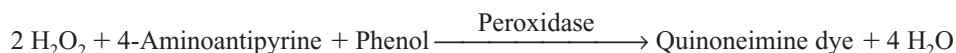
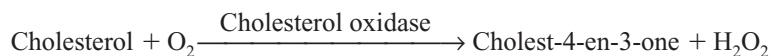
Appearance	yellow lyophilizate
Activity	≥5 U/mg lyophilizate
Contaminants	catalase ≤2.0% cholesterol esterase ≤1.0×10 <sup>-2</sup> %
Stabilizer	trehalose, glutamate
Storage	at -20°C

## PROPERTIES

Molecular weight	ca. 58 kDa (gel filtration)
Structure	monomer of 55 kDa (SDS-PAGE)
Michaelis constant	3.5×10 <sup>-4</sup> M (cholesterol)
pH Optimum	7.0 (Fig. 1)
pH Stability	5.0–10.0 (Fig. 2)
Optimum temperature	50°C (Fig. 3)
Thermal stability	below 55°C (Fig. 4)
Stability (liquid form)	stable at 37°C for at least one week (Fig. 5)
Stability (powder form)	stable at 30°C for at least one month (Fig. 6)
Inhibitors	ionic detergents, Ag <sup>+</sup> , Hg <sup>2+</sup>

## ASSAY PROCEDURE

### Principle



The appearance of quinoneimine dye is measured spectrophotometrically at 500 nm.

### Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1  $\mu\text{mol}$  of hydrogen peroxide per min at 37°C and pH 7.0 under the conditions described below.

### Reagents

- Potassium phosphate buffer, 0.1 M; pH 7.0: mix 0.1 M  $\text{KH}_2\text{PO}_4$  solution and 0.1 M  $\text{K}_2\text{HPO}_4$  solution to make a pH 7.0 solution.
- Cholesterol solution, 0.5%: dissolve 500 mg of cholesterol in 5.0 ml of Triton X-100 with heating. Add 90 ml of distilled water to the hot cholesterol–Triton X-100 solution by slowly pouring. Stir and boil for 30–60 s. Cool under running water with gentle agitation. Dissolve 4.0 g of sodium cholate in the cholesterol–Triton X-100 solution and dilute with distilled water to 100 ml. This solution is stable for about one week at room temperature. If it becomes cloudy, warm slightly with stirring until it becomes clear.
- 4-Aminoantipyrine (4-AA) solution, 1.76%: 1.76 g of 4-AA/100 ml of distilled water.
- Phenol solution, 6.0%: 6.0 g of phenol/100 ml of distilled water.
- Peroxidase (POD) solution, 150 U/ml: 75 mg of POD (200 guaiacol U/mg)/100 ml of potassium phosphate buffer (Reagent A).
- Enzyme dilution buffer: 20 mM potassium phosphate buffer, pH 7.0, containing 0.2% bovine serum albumin (BSA).

Sample: dissolve the lyophilized enzyme to a volume activity of 0.1–0.5 U/ml with ice-cold enzyme dilution buffer (Reagent F).

### Procedure

- Pipette the following reagents into a cuvette (light path: 1 cm).

2.55 ml	Potassium phosphate buffer	(Reagent A)
0.20 ml	Cholesterol solution	(Reagent B)
0.05 ml	4-AA solution	(Reagent C)
0.10 ml	Phenol solution	(Reagent D)
0.10 ml	POD solution	(Reagent E)
- Equilibrate at 37°C for about 5 min.
- Add 0.05 ml of sample and mix.
- Record the increase of absorbance at 500 nm in a spectrophotometer thermostated at 37°C, and calculate the  $\Delta A$  per min using the linear portion of the curve ( $\Delta A_s$ ).  
The blank solution is prepared by adding enzyme dilution buffer (Reagent F) instead of sample ( $\Delta A_0$ ).

**Calculation**

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_s - \Delta A_0) \times 3.05(\text{ml}) \times df}{13.8 \times 1/2 \times 0.05(\text{ml})} = \Delta A \times 8.84 \times df$$

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/C$$

13.8 : Millimolar extinction coefficient of quinoneimine dye under the assay conditions (cm<sup>2</sup>/μmol)

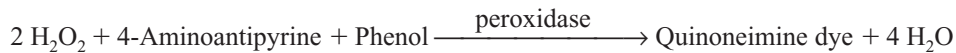
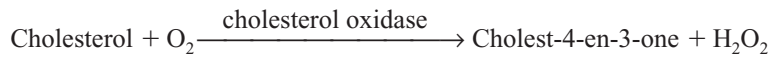
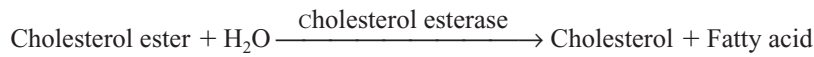
1/2 : Factor based on the fact that 1 mol of hydrogen peroxide produces 1/2 mol of quinoneimine dye

df : Dilution factor

C : Content of cholesterol oxidase preparation in sample (mg/ml)

**APPLICATIONS**

The enzyme is useful for the determination of cholesterol in clinical analysis.



**REFERENCES**

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 Flegg, H. M., *Ann. Clin. Biochem.*, **10**, 79–84 (1973).  
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## EXPERIMENTAL DATA

Fig. 1 pH Optimum

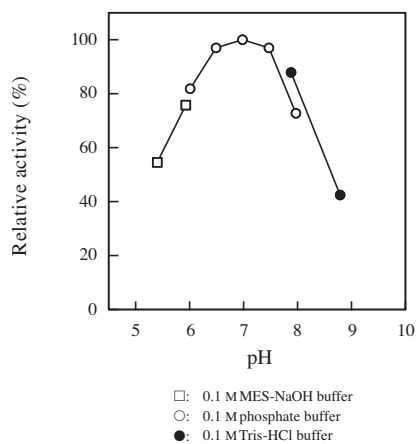


Fig. 2 pH Stability

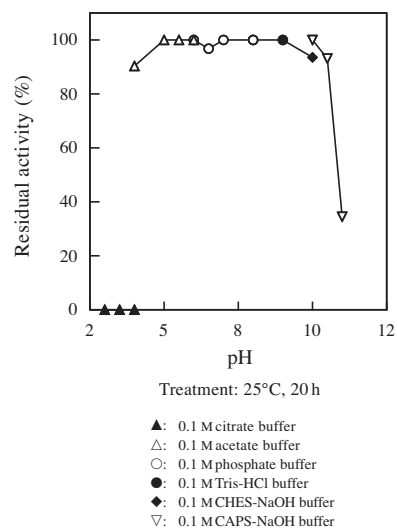


Fig. 3 Optimum temperature

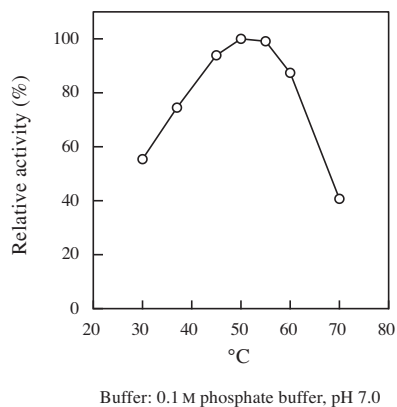


Fig. 4 Thermal stability

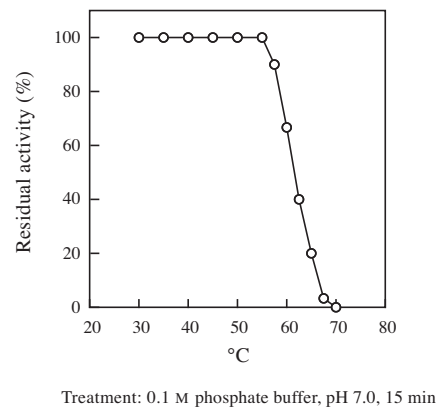


Fig. 5 Stability (liquid form) at 5°C and 37°C

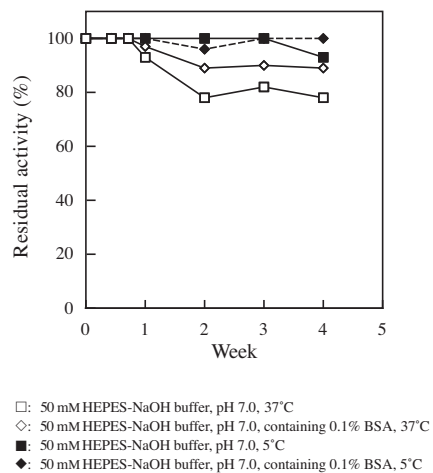


Fig. 6 Stability (powder form) at 30°C

