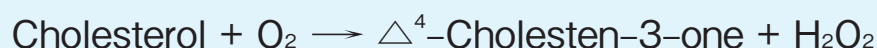


# CHOLESTEROL OXIDASE [CON II]

## Liquid type

from *Rhodococcus* sp.

(Cholesterol: oxygen oxidoreductase, EC 1.1.3.6)



### Preparation and Specification

Appearance : Yellowish solution  
Specific activity : More than 500 U/ml

### Properties

Substrate specificity	: See Table 1	
Molecular weight	: 61.8 KDa (SDS-PAGE)	
Isoelectric point	: pH 4.5	
Michaelis constant	: Cholesterol $6.0 \times 10^{-5}\text{M}$	
Optimum pH	: 7.0-7.5	Figure 1
pH stability	: 5.7-7.8 (65°C, 10 min)	Figure 2
Optimum temperature	: 50°C (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 65°C and below (pH 7.0, 10 min)	Figure 4 and Figure 5
Effects of detergents	: See Table 2	

### Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **total cholesterol**, **HDL-C**, and **LDL-C** when coupled with cholesterol esterase (T-18 and T-98).

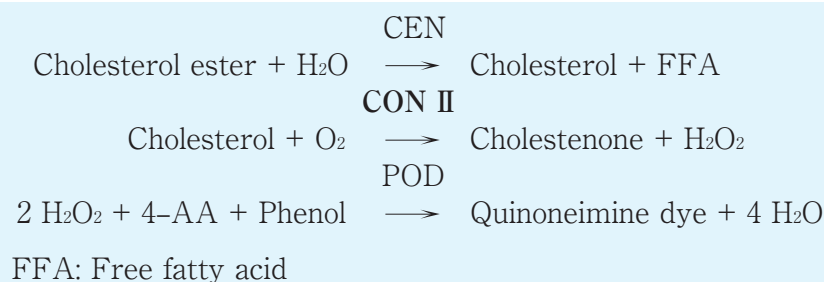


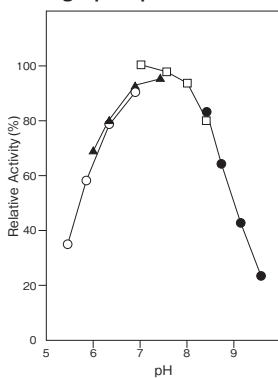
Table 1. Substrate specificity

Substrate (1mM)	Relative activity (%)
Cholesterol	100
$\beta$ -Cholesterol	93
Pregnenolone	98
Dehydro-iso-androsterone	10
$\beta$ -Sitosterol	94
Stigmasterol	66
Androsterol	2
Teststerone	1
Cholic acid	3

Table 2. Effect of detergents on CON II activity

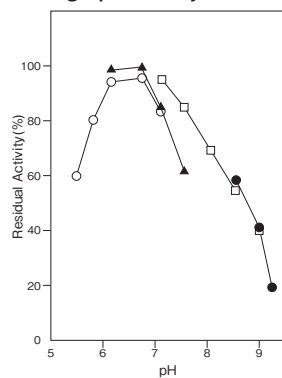
Detergents (0.1%)	Relative activity (%)
Triton X-100	100
Emulgen 810	101
Emulgen 911	113
Emulgen 709	107
Emulgen 109P	118
Adekamol SO-120	100
RHEODOL 460	63
SM 1080	122

Fig.1 pH Optimum



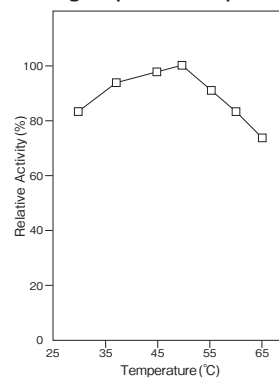
○ : MES buffer  
 ▲ : PIPES buffer  
 □ : Tris-HCl buffer  
 ● : Glycine-NaOH buffer

Fig.2 pH Stability



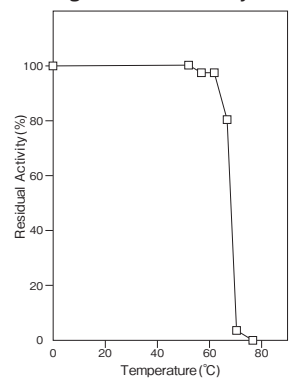
65°C, 10 min.  
 ○ : MES buffer  
 ▲ : PIPES buffer  
 □ : Tris-HCl buffer  
 ● : Glycine-NaOH buffer

Fig.3 Optimum Temperature



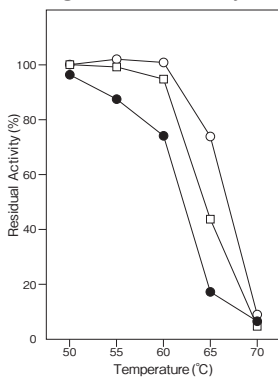
pH 7.0  
 100 mM Tris-HCl buffer

Fig.4 Thermal Stability



pH 7.0, 10 min.  
 100 mM Tris-HCl buffer

Fig.5 Thermal Stability

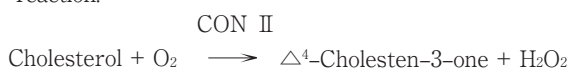


100mM, 10min.  
 ○ : MES buffer(pH5.95)  
 □ : Tris-HCl buffer(pH7.63)  
 ● : Glycine-NaOH buffer(pH8.95)

## Assay

### Principle

The assay is based on the increase in absorbance at 240 nm as  $\Delta^4$ -cholesten-3-one is produced in the following reaction:



### Unit definition

One unit is defined as the amount of enzyme which liberates 1  $\mu$ mole of  $\Delta^4$ -cholesten-3-one per minute at 37°C under the conditions specified in the assay procedure.

### Reagents

- Substrate solution (6 mM cholesterol solution)  
 Dissolve 232 mg of cholesterol with isopropanol to make a total of 100 ml.

2. Enzyme dilution buffer  
0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  buffer pH 7.0 containing  
0.05% (W/V) Triton X-100
3. Reagents  
Cholesterol : NACALAI TESQUE, INC. Special grade  
#08721  
Triton X-100 : The Dow Chemical Company

#### ■ Enzyme solution

Dilute accurately 0.5 ml of the sample with enzyme dilution buffer to make a 40-fold solution. Dilute it with enzyme dilution buffer to adjust the concentration to within 0.1-0.2 U/ml.

#### ■ Procedure

- Pipette accurately 3.0ml of enzyme dilution buffer and 50  $\mu\text{l}$  of enzyme solution and preincubate at 37°C.  
※ In the case of a test blank, add 50  $\mu\text{l}$  of enzyme dilution buffer in place of enzyme solution.
- After 5 min, add 50  $\mu\text{l}$  of substrate solution and mix to start the reaction at 37°C.
- After starting the reaction, measure the rate of increase per minute in absorbance at 240nm. The rate must be measured within the linear portion of the absorbance curve.

Absorbance sample : As/min  
blank : Ab/min  
 $0.010 \text{ Abs/min} \leq \Delta A/\text{min} = (\text{As/min}-\text{Ab/min})$   
 $\leq 0.060 \text{ Abs/min}$

#### ■ Calculation

$$\text{Activity (U/ml)} = \frac{\Delta A/\text{min}}{12.2} \times \frac{3.10}{0.05} \times D$$

- 12.2 : millimolar extinction coefficient of  $\Delta^4$ -Cholesten-3-one at 240 nm ( $\text{cm}^2 / \mu\text{mole}$ )  
3.10 : final volume (ml)  
0.05 : volume of enzyme solution (ml)  
D : times of dilution in enzyme solution

#### Storage

Storage at -80°C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition.

#### References

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- Tarbutton, P. N. and Gunter, C. R. (1974) Clin. Chem., **20**, 724.
- Nomoto, S. (1976) Rinsho Kensa, **20**, 688.
- Kameno, K., Nakano, N. and Baba, S. (1976) Jap. J. Clin. Path., **24**, 650.

## CON II 活性測定法 (Japanese)

### I. 試薬液

- 基質溶液 (6mM コレステロール溶液)  
コレステロール 232mg をイソプロパノールに溶解して全容 100ml とする。
- 酵素溶解希釈用液  
0.05% (W/V) トリトン X-100 を含む  
0.1M  $\text{KH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  緩衝液 pH7.0
- 試薬  
コレステロール : ナカライテスク製 特級 #08721  
トリトン X-100 : Dow Chemical 製

### II. 酵素試料液

検品 0.5ml を酵素溶解希釈用液で 40 倍に希釈する。その液を酵素溶解希釈用液で約 0.1-0.2U/ml 濃度となるように適宜希釈する。

### III. 測定操作法

- 小試験管に酵素溶解希釈用液 3.0ml と酵素試料液 50  $\mu\text{l}$  を正確に加え 37°C で予備加温する。  
※盲検は酵素試料液の代わりに酵素溶解希釈用液 50  $\mu\text{l}$  を加える。

- 5分経過後、基質溶液 50  $\mu\text{l}$  を正確に加えて混和し、37°C で反応を開始する。
- 反応開始後、240nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光変化を求める。求められた吸光度変化を試料液は As/min, 盲検液は Ab/min とする。  
 $0.010 \text{ Abs/min} \leq \Delta A/\text{min} = (\text{As/min}-\text{Ab/min})$   
 $\leq 0.060 \text{ Abs/min}$

### IV. 計算

$$\text{活性 (U/ml)} = \frac{\Delta A/\text{min}}{12.2} \times \frac{3.10}{0.05} \times D$$

- 12.2 :  $\Delta^4$ -コレステレン-3-オンの 240nm におけるミリモル分子吸光係数 ( $\text{cm}^2 / \mu\text{mole}$ )  
3.10 : 反応総液量 (ml)  
0.05 : 反応に供した酵素試料液量 (ml)  
D : 酵素試料液の希釈倍率