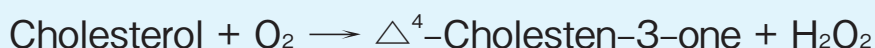


CHOLESTEROL OXIDASE [CON II -FD]

Lyophilized type

from *Rhodococcus* sp.
(Cholesterol: oxygen oxidoreductase, EC 1.1.3.6)



Preparation and Specification

Appearance : Yellowish amorphous powder, lyophilized
Specific activity : More than 15 U/mg solid

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).

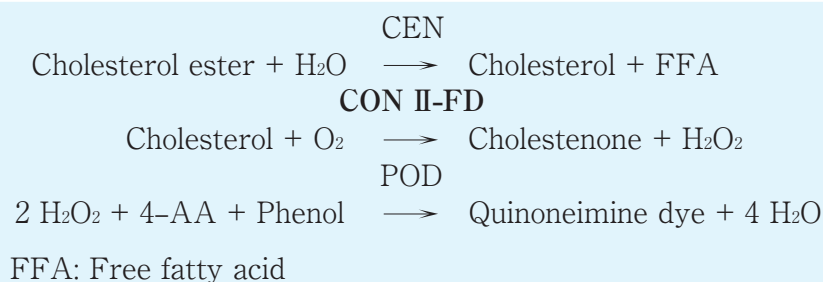


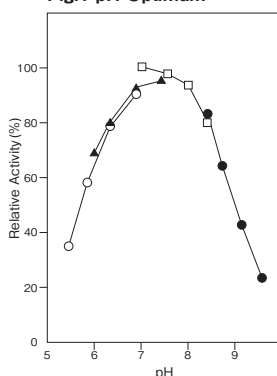
Table 1. Substrate specificity

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

Table 2. Effect of detergents on CON II -FD activity

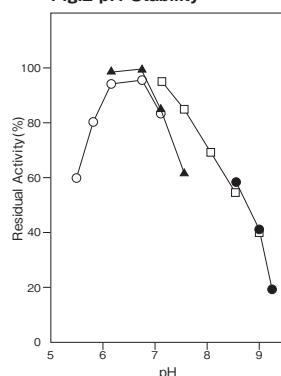
Detergents (0.1%)	Relative activity (%)
Triton X-100	100
Emulgen 810	101
Emulgen 911	113
Emulgen 709	107
Emulgen 109P	118
AdekatoI SO-120	100
RHEODOL 460	63
SM 1080	122

Fig.1 pH Optimum



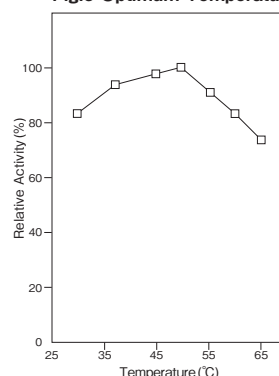
○ : MES buffer
 ▲ : PIPES buffer
 □ : Tris-HCl buffer
 ● : Glycine-NaOH buffer

Fig.2 pH Stability



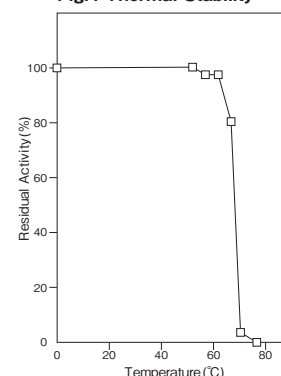
65°C, 10 min.
 ○ : MES buffer
 ▲ : PIPES buffer
 □ : Tris-HCl buffer
 ● : Glycine-NaOH buffer

Fig.3 Optimum Temperature



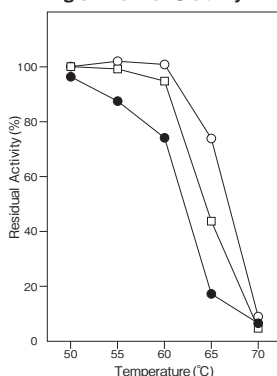
pH 7.0
 100 mM Tris-HCl buffer

Fig.4 Thermal Stability



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

Fig.5 Thermal Stability

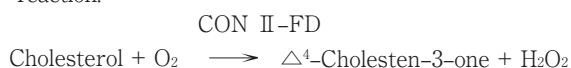


100mM, 10min.
 ○ : MES buffer(pH5.95)
 □ : Tris-HCl buffer(pH7.63)
 ● : Glycine-NaOH buffer(pH8.95)

Assay

Principle

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



Unit definition

One unit is defined as the amount of enzyme which liberates 1 μmole of Δ^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 KH₂PO₄-Na₂HPO₄ buffer pH 7.0 containing
0.05% (W/V) Triton X-100
※ Prepare the enzyme dilution buffer two days before
use and keep it in the refrigerator until use.
3. Reagents
Cholesterol : NACALAI TESQUE, INC. Special grade
#08721
Triton X-100 : The Dow Chemical Company

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. After 1-1.5 hour incubation at room temperature, 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 μl of enzyme solution and preincubate at 37°C.
※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
- After 5 min, add 50 μl 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

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

■ Calculation

$$\text{Activity (U/mg)} = \frac{\Delta A/\text{min}}{12.2} \times \frac{3.10}{0.05} \times \frac{1}{X}$$

- 12.2 : millimolar extinction coefficient of Δ^4 -Cholesten-3-one at 240 nm (cm² / μmole)
3.10 : final volume (ml)
0.05 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at -20°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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CON II-FD 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 KH₂PO₄-Na₂HPO₄ 緩衝液 pH7.0
※酵素溶解希釈用液は使用する 2 日前に調製し、使用まで冷蔵保存する。
- 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

検品約 20mg を精密に量り、酵素溶解希釈用液で溶解して全容 20ml とする。室温にて 1~1.5 時間放置し、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 μl 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 μl を加える。

- 5 分経過後、基質溶液 50 μl 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。

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

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min}}{12.2} \times \frac{3.10}{0.05} \times \frac{1}{X}$$

- 12.2: Δ^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 (cm² / μmole)
3.10: 反応総液量 (ml)
0.05: 反応に供した酵素試料液量 (ml)
X : 酵素試料液の検品濃度 (mg/ml)