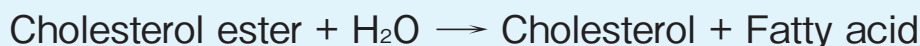


CHOLESTEROL ESTERASE [CEN]

from *Pseudomonas* sp.
(Steryl-ester acylhydrolase, EC 3.1.1.13)
(Sterol esterase)



Preparation and Specification

Appearance : White to pale brownish amorphous powder, lyophilized
Specific activity : More than 100 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 29.5 kDa (SDS-PAGE)	
	: 31.0 kDa (Sephadex G-100)	
Isoelectric point	: pH 4.25	
Michaelis constants	: Cholesterol linolate	$1.28 \times 10^{-3}\text{M}$
	: Cholesterol ester of calf serum	$7.5 \times 10^{-4}\text{M}$
Optimum pH	: 6.5	Figure 1
pH stability	: 6.5–10.0 (37°C, 60 min)	Figure 2
Thermal stability	: Stable at 55°C and below (pH 8.0, 10 min)	Figure 3
Storage stability	: At least one year at -20°C	Figure 4
Activator	: Triton X-100	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **total cholesterol**, **HDL-C**, and **LDL-C** coupled with cholesterol oxidase T-84 and T-101.

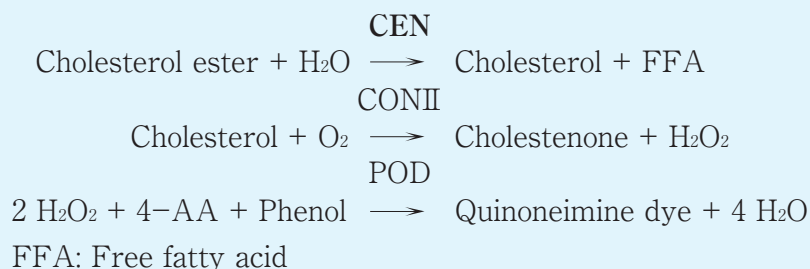
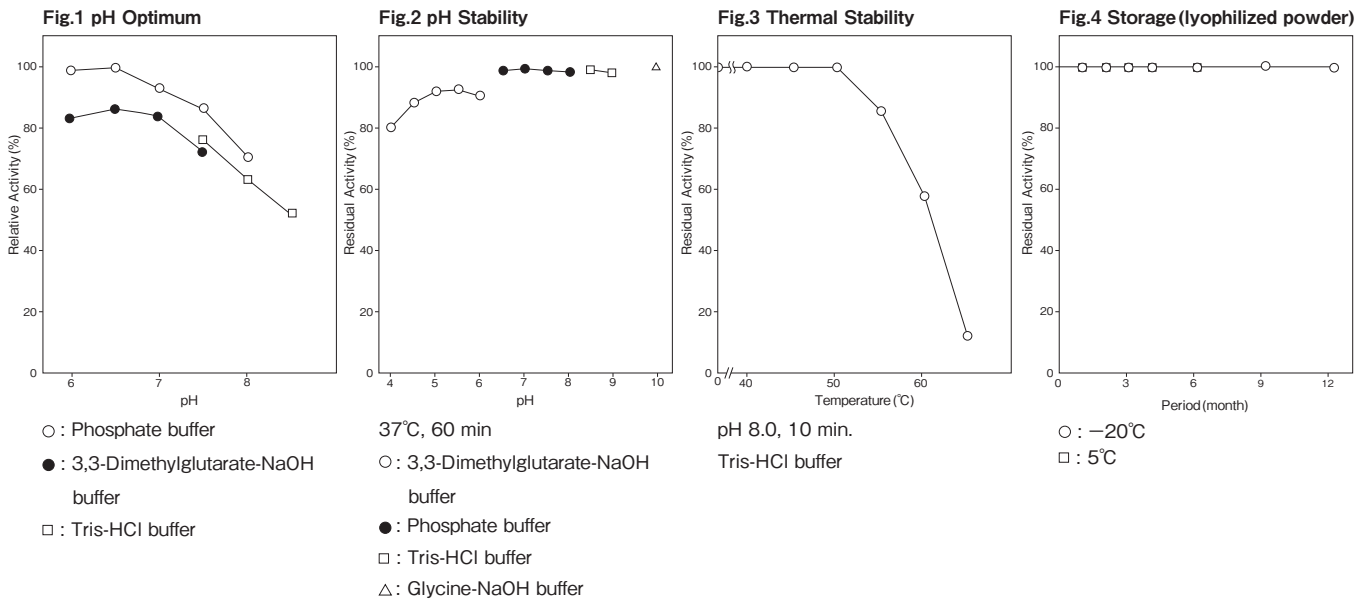


Table 1. Substrate specificity

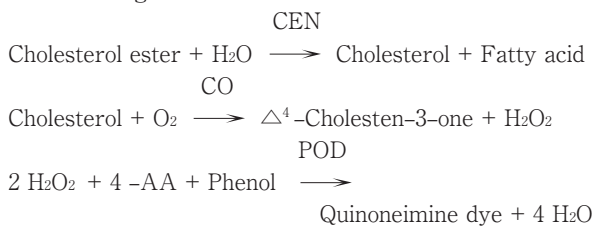
Substrate	Relative activity (%)
Cholesterol acetate C 2:0	3.2
propionate 3:0	12.3
butylate 4:0	26.7
palmitate 16:0	25.7
stearate 18:0	14.9
oleate 18:1	100.0
linoleate 18:2	534.8



Assay

Principle

The assay is based on the increase in absorbance at 493 nm as the formation of quinoneimine dye proceeds in the following reactions:



CO: Cholesterol oxidase

Unit definition

One unit is defined as the amount of enzyme which liberates 1 μmole of cholesterol per minute at 37°C under the conditions specified in the assay procedure.

Reagents

1. Reaction mixture		
0.2M $\text{KH}_2\text{PO}_4\text{-NaOH}$ buffer pH 6.8		0.60 ml
0.35% (W/V) 4-AA solution		0.30 ml
0.2% (W/V) Phenol solution		0.30 ml
100 U/ml POD solution ¹⁾		0.30 ml
3% (W/V) Triton X-100 solution		0.30 ml
0.2 U/ml CONII solution ²⁾		0.60 ml

Substrate solution ³⁾	0.30 ml
Distilled water	0.30 ml

1) : 100 U/ml POD solution

Dissolve 1000 U (PPU) of POD with 10 ml of distilled water.

2) : 0.2 U/ml CONII solution

Dissolve 2 U of CONII with CONII dilution buffer ^{**)}

*) : CONII 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) : Substrate solution

Calf serum

2. Enzyme dilution buffer

10 mM $\text{KH}_2\text{PO}_4\text{-NaOH}$ buffer pH 7.5 containing 0.1% (W/V) bovine serum albumin (BSA) .

3. Reagents

Triton X-100: The Dow Chemical Company

CONII : Asahi Kasei Pharma Corporation #T-84

Calf serum: GIBCO Co. (USA)

BSA: Millipore Fraction V pH 5.2 #81-053

4-AA: NACALAI TESQUE, INC. Special grade #01907-52

POD: Sigma Chemical Co. Type II #P-8250

Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration to within 0.3-0.5 U/ml.

■ Procedure

- Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate it at 37°C.
- After 10 min, add 50 μ l of enzyme solution and mix to start the reaction at 37°C.
※ In the case of a test blank, add 50 μ l of enzyme dilution buffer in place of enzyme solution.
- After starting the reaction, measure the rate of increase per minute in absorbance at 493 nm. The rate must be measured within the linear portion of the absorbance curve.

$$\begin{aligned} \text{Absorbance sample} &: \text{As/min} \\ \text{blank} &: \text{Ab/min} \\ \Delta A/\text{min} &= (\text{As/min} - \text{Ab/min}) \leq 0.050 \text{ Abs/min} \end{aligned}$$

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A/\text{min}}{12.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

- 12.0 : millimolar extinction coefficient of quinoneimine dye at 493 nm ($\text{cm}^2/\mu\text{mole}$)
 1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produce 1 mole of quinoneimine dye
 3.05 : 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 (Figure 4).

References

- Bradford, M. B., (1976) Anal. Biochem., **72**, 248-254.
- Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P.C. (1974) Clin. Chem., **20**, 470-475.
- Kameno, Y., Nakano, N. and Baba, S. (1976) Japanese Journal of Clinical Pathology, **24**, 650.

CEN 活性測定法 (Japanese)

I. 試薬液

1. 反応試薬混合液		
0.2M KH_2PO_4 -NaOH 緩衝液 pH6.8		0.60 ml
0.35% (W/V) 4-AA 溶液		0.30 ml
0.2% (W/V) フェノール溶液		0.30 ml
100U/ml POD 溶液 ¹⁾		0.30 ml
3% (W/V) トリトン X-100 溶液		0.30 ml
0.2U/ml CONII 溶液 ²⁾		0.60 ml
基質溶液 ³⁾		0.30 ml
精製水		0.30 ml

- 1): 100U/ml POD 溶液
 POD 1,000 単位 (PPU) を精製水 10ml で溶解する。
 2): 0.2U/ml CONII 溶液
 CONII 2 単位 (U) を CONII 溶解用液^{※)} 10ml で溶解する。
 ※): CONII 溶解用液
 0.05% (W/V) トリトン X-100 を含む 0.1M KH_2PO_4 - Na_2HPO_4 緩衝液 pH7.0

- 3): 基質溶液
 仔牛血清液

2. 酵素溶解希釈用液
 0.1% (W/V) BSA を含む 10mM KH_2PO_4 -NaOH 緩衝液 pH7.5

3. 試薬
 トリトン X-100 : Dow Chemical 製
 CONII (コレステロール酸化酵素) :
 旭化成ファーマ製 #T-84
 仔牛血清液 (Calf serum) : GIBCO (USA) 製
 BSA: Millipore 製 Fraction V pH5.2 #81-053

4-AA: ナカライテスク製 特級 #01907-52
 POD: シグマ製 Type II #P-8250

II. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。
 その液を酵素溶解希釈用液で 0.3~0.5U/ml 濃度となるように適宜希釈する。

III. 測定操作法

- 小試験管に反応試薬混合液を 3.0ml 正確に分注して 37°C で予備加温する。
- 10 分経過後、酵素試料液 50 μ l を正確に加えて混和し、37°C で反応を開始する。
 ※盲検は酵素試料液の代わりに酵素溶解希釈用液 50 μ l を加える。
- 反応開始後、493nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求めらる。
 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。
 $\Delta A/\text{min} = (\text{As/min} - \text{Ab/min}) \leq 0.050 \text{ Abs/min}$

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min}}{12.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

- 12.0 : キノンイミン色素の 493nm におけるミリモル分子吸光係数 ($\text{cm}^2/\mu\text{mole}$)
 1/2 : H_2O_2 2 モルからキノンイミン色素 1 モルが生成することによる係数
 3.05 : 反応総液量 (ml)
 0.05 : 反応に供した酵素試料液量 (ml)
 X : 酵素試料液中の検品濃度 (mg/ml)