

(Diagnostic Reagent Grade)

T-243

CHOLESTEROL ESTERASE [CEBP II]

from Microorganism
(Sterol-ester acylhydrolase, EC 3.1.1.13)
(Sterol esterase)



★ Advantage
High liquid stability

Preparation and Specification

Appearance : White to off white lyophilized powder
Specific activity : More than 10.0 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 63 kDa (SDS-PAGE)	
Isoelectric point	: 5.1 (estimated from amino acid sequence)	
Optimum pH	: 5.6	Figure 1
pH stability	: 4.0-8.0 (37°C, 60 min)	Figure 2
Optimum temperature	: 45°C	Figure 3
Thermal stability	: Stable at 45°C and below (pH6.0, 30 min)	Figure 4
Liquid stability	: See Figure 5	
Adsorption on glass	: See Figure 6	
Effect of coexisting ions	: See Table 2	
Effect of detergents	: See Table 3	
Light stability	: See Table 4	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **total cholesterol**, **HDL-C**, and **LDL-C** coupled with cholesteryl oxidase (T-84 and T-101).
This enzyme is suitable for assembling in liquid reagents.

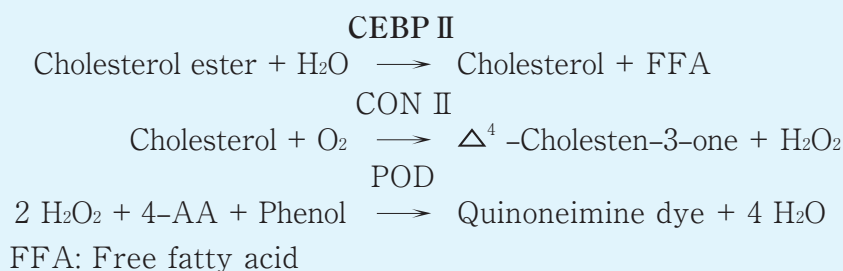


Table 1 Substrate specificity

Substrate (0.95mM)	Relative activity (%)
Cholesterol Acetate C 2:0	0.4
Cholesterol Propionate C 3:0	9.3
Cholesterol Butyrate C 4:0	27.8
Cholesterol Palmitate C16:0	19.8
Cholesterol Stearate C18:0	2.0
Cholesterol Oleate C18:1	100.0
Cholesterol Linoleate C18:2	80.6

Table 2 Effect of coexisting ions on CEBP II activity

Coexisting ion (20 mM)	Relative activity (%)
None	100
NaCl	101
KCl	100
NH ₄ Cl	100
CaCl ₂	101
MgCl ₂	101
MnCl ₂	103
ZnCl ₂	105
EDTA	102
NaN ₃	100
NaF	98

All samples contain 0.1% BSA

Table 3 Effect of detergents on CEBP II activity

Detergent (0.1%)	Relative activity (%)
None	100
Triton X-100	115
AdekatoI TN-100	117
AdekatoI SO-120	142
Newcol-707	115
Newcol-710	113
Emulgen 120	141
Emulgen 705	135
Deoxycholic acid	129
Tauroursodeoxycholic acid	134
Sodium dodecyl sulfate	127

Table 4 Light stability

Form of CEBP II	Light source	Residual activity (%)
Powder	LED	96.5
	Fluorescent	98.7
Solution (1mg/ml, water)	LED	94.1
	Fluorescent	98.4

After 24 hours of incubation

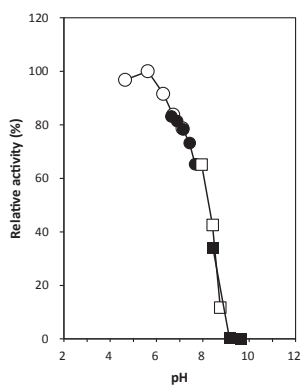


Fig. 1 Optimum pH

○ : 3,3-Dimethylglutarate-NaOH buffer
● : Phosphate buffer
□ : Tris-HCl buffer
■ : Glycine-NaOH buffer

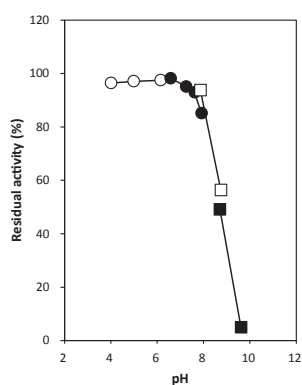


Fig. 2 pH stability

37°C, 60 min
○ : 3,3-Dimethylglutarate-NaOH buffer
● : Phosphate buffer
□ : Tris-HCl buffer
■ : Glycine-NaOH buffer

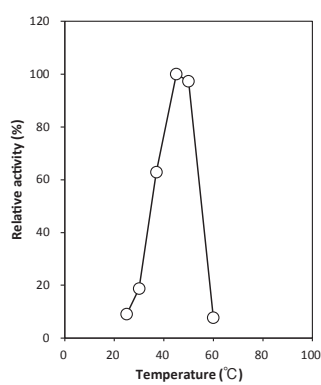


Fig. 3 Optimum temperature

pH 7.5
40 mM Phosphate buffer

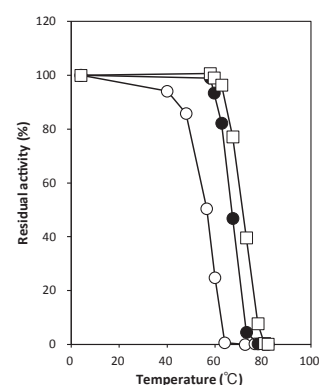


Fig. 4 Temperature stability

pH 6.0, 30 min
○ : Phosphate buffer
● : Phosphate buffer + 0.1% Emulgen 705
□ : Phosphate buffer + 0.1% Emulgen 705 & 10 mM NH₄Cl

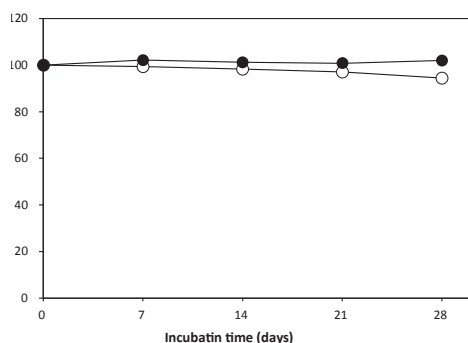


Fig. 5 Stability of CEBP II in a liquid reagent

● : 4°C Reagent composition: 0.5 U/mL CEBP II
○ : 37°C 50 mM BES buffer pH 6.5
0.4% Triton X-100
0.05% NaN₃
0.6 mM ADOS
7.5 U/mL POD

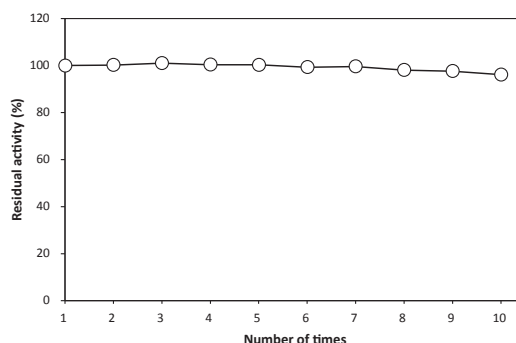


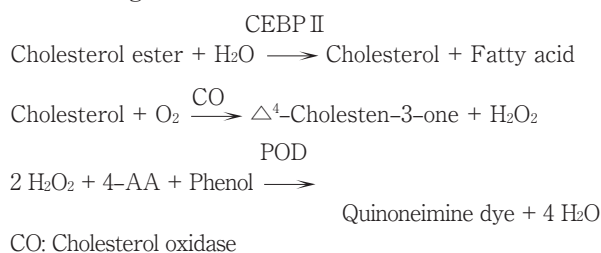
Fig. 6 Adsorption on glass

pH 7.0
10 mM Phosphate buffer containing 0.1% BSA
Solution of CEBP II was repeatedly transferred into another test tube, and then the residual activity in the solution of each tube was measured.

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:



■ 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

- Reaction mixture

0.2M KH ₂ PO ₄ -NaOH buffer pH 6.8	0.60 ml
0.35% 4-AA solution	0.30 ml
0.2% (W/V) Phenol solution	0.30 ml
100U/ml POD solution ¹⁾	0.30 ml
3% (W/V) Triton X-100 solution	0.30 ml
0.2U/ml CONII solution ²⁾	0.60 ml
Substrate solution ³⁾	0.30 ml
Distilled water	0.30 ml

 - 100U/ml POD solution
Dissolve 1000 U (PPU) of POD with 10 ml of distilled water.
 - 0.2U/ml CONII solution
Dissolve 2 U of CONII with CONII dilution buffer ^{*)}
^{*)}: CONII dilution buffer
0.1M KH₂PO₄-Na₂HPO₄ buffer pH 7.0 containing 0.05% (W/V) Triton X-100.
 - Substrate solution
Calf serum

2. Enzyme dilution buffer

10mM KH₂PO₄-NaOH buffer pH 7.5 containing 0.1% (W/V) BSA.

3. Reagents

Triton X-100: The Dow Chemical Company

CONII : Nagase Diagnostics Co., Ltd. #T-84

Calf serum: GIBCO Co. (USA)

BSA: Millipore Fraction V pH5.2 #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 0.35 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 μl of enzyme solution and mix to start the reaction at 37°C.
※ In the case of a test blank, add 50 μl 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.

Absorbance sample : As/min
 blank : Ab/min

$$\Delta A/\text{min} = (A_s/\text{min} - A_b/\text{min}) \leq 0.040 \text{ Abs/min}$$

■ 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 (cm²/ μmole)

1/2 : a multiplier derived from the fact that 2 mole of H₂O₂ 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.

References

1. Bradford, M. B., (1976) Anal. Biochem., **72**, 248-254.
2. Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P.C. (1974) Clin. Chem., **20**, 470-475.
3. Kamen, Y., Nakano, N. and Baba, S. (1976) Jap. J. Clin. Path., **24**, 650.

CEBP II 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% 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 CON II 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 CON II 溶液

CON II 2 単位 (U) を CON II 溶解用液^{*)} 10ml で溶解する。

※): CON II 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 社製

CON II (コレステロール酸化酵素):

ナガセダイアグノスティックス製 #T-84

仔牛血清液 (Calf serum): GIBCO (USA) 製

BSA: Millipore 製 Fraction V pH5.2 #81-053

POD: シグマ製 Type II #P-8250

4-AA: ナカライテスク製 特級 #01907-52

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約 0.35U/ml 濃度となるように適宜希釈する。

III. 測定操作法

1. 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。

2. 10 分経過後、酵素試料液 50 μl 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 μl を加える。

3. 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。

$\Delta A/\text{min} = (\text{As}/\text{min} - \text{Ab}/\text{min}) \leq 0.040 \text{ Abs}/\text{min}$

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 : キノニン色素の 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 モルからキノニン色素 1 モルが生ずることによる係数

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)