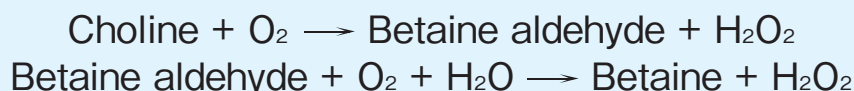


CHOLINE OXIDASE [COD]

from *Arthrobacter globiformis*
(Choline: oxygen 1-oxidoreductase, EC 1.1.3.17)



Preparation and Specification

Appearance	: Yellowish amorphous powder, lyophilized
Specific activity	: More than 8 U/mg solid
Contaminants	:
Catalase	Less than 10.0 % (U/U)
Glucose oxidase	Less than 0.01 % (U/U)

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 83 kDa (Sephadex G-150)	
Isoelectric point	: pH 4.5	
Michaelis constants	: Choline $1.2 \times 10^{-3}\text{M}$ Betaine aldehyde $8.7 \times 10^{-3}\text{M}$	
Optimum pH	: 7.5–8.0	Figure 1
pH stability	: 7.5–9.0 (37°C, 30 min)	Figure 2
Thermal stability	: Stable at 40°C and below (pH 7.5, 10 min)	Figure 3
Storage stability	: At least one year at -20°C	Figure 4
Effect of various chemicals	: See Table 2	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **phospholipids** coupled with phospholipase D (T-07).

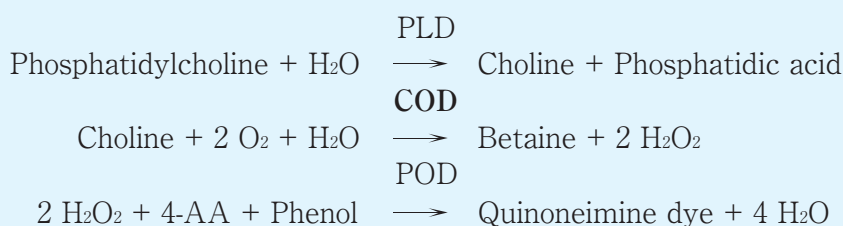


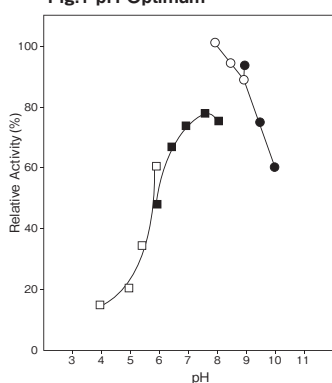
Table 1. Substrate specificity

Substrate	Relative activity (%)
Choline	100
Betaine aldehyde	46.2
Diethanolamine	0.8
Triethanolamine	2.6
N, N-Dimethylaminoethanol	5.2
N-Methylethanolamine	0

Table 2. Effect of various chemicals on COD activity

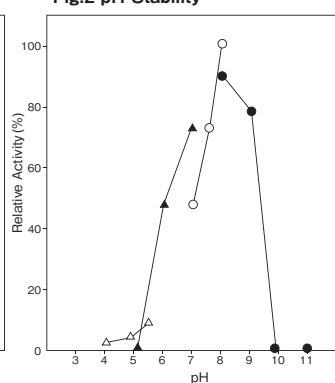
Additives	Concentration	Relative activity (%)
None	-	100
Triton X-100	0.1%	95.5
Adekamol SO-120	0.1	105.7
Sodium laurylsulfate	0.1	94.3
Deoxycholate	0.1	94.3
Sodium laurylbenzene-sulfate	0.1	91.0
CaCl ₂	5mM	101.0
MgCl ₂	5	100.0
FeCl ₃	5	0
ZnCl ₂	5	7.9
MnCl ₂	5	97.7
CoCl ₂	5	30.7
MoCl ₂	5	58.0
KCl	5	93.2
NaCl	5	96.6
NH ₄ Cl	5	100.0
LiCl	5	96.6
BaCl ₂	5	103.4

Fig.1 pH Optimum



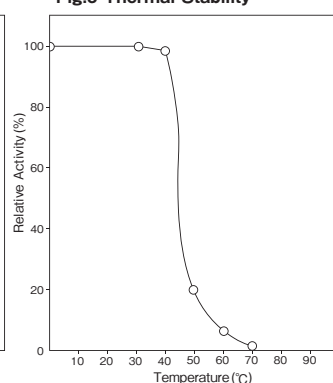
□ : Dimethylglutarate-NaOH buffer
 ■ : Phosphate buffer
 ○ : Glycylglycine buffer
 ● : Glycine-NaOH buffer

Fig.2 pH Stability



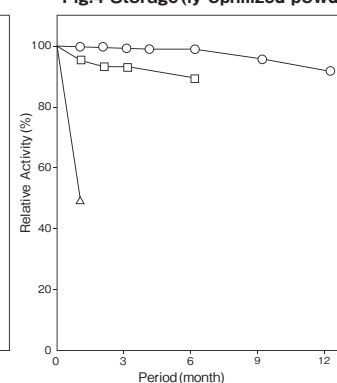
37°C, 10 min
 △ : Acetate buffer
 ▲ : Dimethylglutarate-NaOH buffer
 ○ : Tris-HCl buffer
 ● : Glycine-NaOH buffer

Fig.3 Thermal Stability



pH 7.5, 10 min

Fig.4 Storage (lyophilized powder)

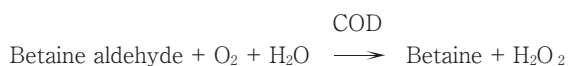


○ : -20°C
 □ : 5°C
 △ : 30°C

Assay

Principle

The assay is based on the increase in absorbance at 500 nm as the formation of quinoneimine dye proceeds in the following reactions:



Unit definition

One unit is defined as the amount of enzyme which generates 1 μmole of H_2O_2 per minute at 37°C under the conditions specified in the assay procedure.

Reagents

1. Reaction mixture

1.211 g of Tris (hydroxymethyl) amino methane, 2.1 g of choline chloride and 2 ml of 1 % (W/V) phenol are dissolved with 1 N HCl and adjusted to pH 8.0 (25°C). Then, 1 ml of 1 % (W/V) 4-AA and 3 ml of 100 PPU/ml POD are added to make a total of 100 ml.

2. Enzyme dilution buffer

10 mM Tris-HCl buffer (pH 8.0) containing 2 mM EDTA and 1 % (W/V) KCl
 EDTA: Ethylenediaminetetraacetic acid

3. Reagents

Choline chloride: Wako Pure Chemical Industries, Ltd.
 (for biochemistry) #033-09812
 4-AA: NACALAI TESQUE, INC. Special grade #01907-52
 POD: Sigma Chemical Co. Type II #P-8250
 EDTA (2 Na·2H₂O) : KISHIDA CHEMICAL Co., Ltd.

#060-29133

■ 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 as required.

■ Procedure

1. Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate it at 37°C.
2. After 5 min, add 50 μ l of enzyme solution and mix to start the reaction at 37°C.
3. After starting the reaction, measure the rate of increase per minute in absorbance at 500 nm. The rate must be measured within the linear portion of the absorbance curve.

$$\Delta A/\text{min} \leq 0.040 \text{ Abs}/\text{min}$$

■ 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 500 nm ($\text{cm}^2/\mu\text{mole}$)

1/2 : 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).

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COD 活性測定法 (Japanese)

I. 試薬液

1. 反応試薬混合液

トリス (ヒドロキシメチル) アミノメタン 1.211g と塩化コリン 2.1g 及び 1% (W/V) フェノール液 2ml を精製水に溶解した後、1N HCl で pH8.0 (25°C) に調整し、さらに 1% (W/V) 4-AA 溶液 1ml と 100PPU/ml POD 溶液 3ml を加えて溶かし、全容 100ml とする。

2. 酵素溶解希釈用液

2mM EDTA と 1% (W/V) KCl を含む 10mM トリス-HCl 緩衝液 pH8.0 溶液

3. 試薬

塩化コリン: 和光純薬工業製 生化学用 #033-09812
4-AA: ナカライテスク社製 特級 #01907-52
POD: シグマ社製 Type II #P-8250
EDTA (エチレンジアミン四酢酸 \cdot 2Na \cdot 2H $_2$ O): キシダ化学社製 #060-29133

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。

その液を酵素溶解希釈用液で適宜希釈する。

III. 測定操作法

1. 小試験管に反応試薬混合液 3.0ml を正確に分注し 37°C で予備加温する。
2. 5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。
3. 反応開始後、500nm 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。

$$\Delta A/\text{min} \leq 0.040 \text{ Abs}/\text{min}$$

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 : キノンイミン色素の 500nm におけるミリモル分子吸光度 ($\text{cm}^2/\mu\text{mole}$)

1/2 : H_2O_2 2モルからキノイミン色素 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 : 酵素試料液の検品濃度 (mg/ml)