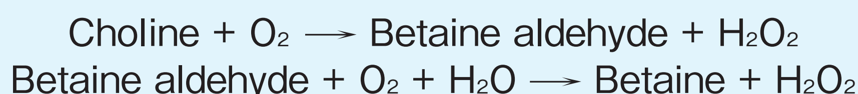
CHOLINE OXIDASE [COD]

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 1.2×10^{-3}M	
	Betaine aldehyde 8.7×10^{-3}M	
Optimum pH	: 7.5–8.0	Figure 1
pH stability	: 7.5–9.0 (37℃, 10 min)	Figure 2
Thermal stability	: Stable at 40℃ and below (pH 7.5, 10 min)	Figure 3
Storage stability	: At least one year at –20℃	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).

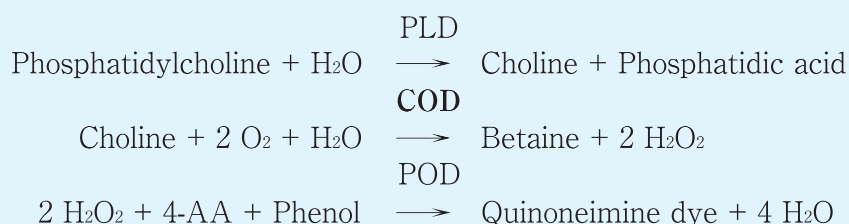
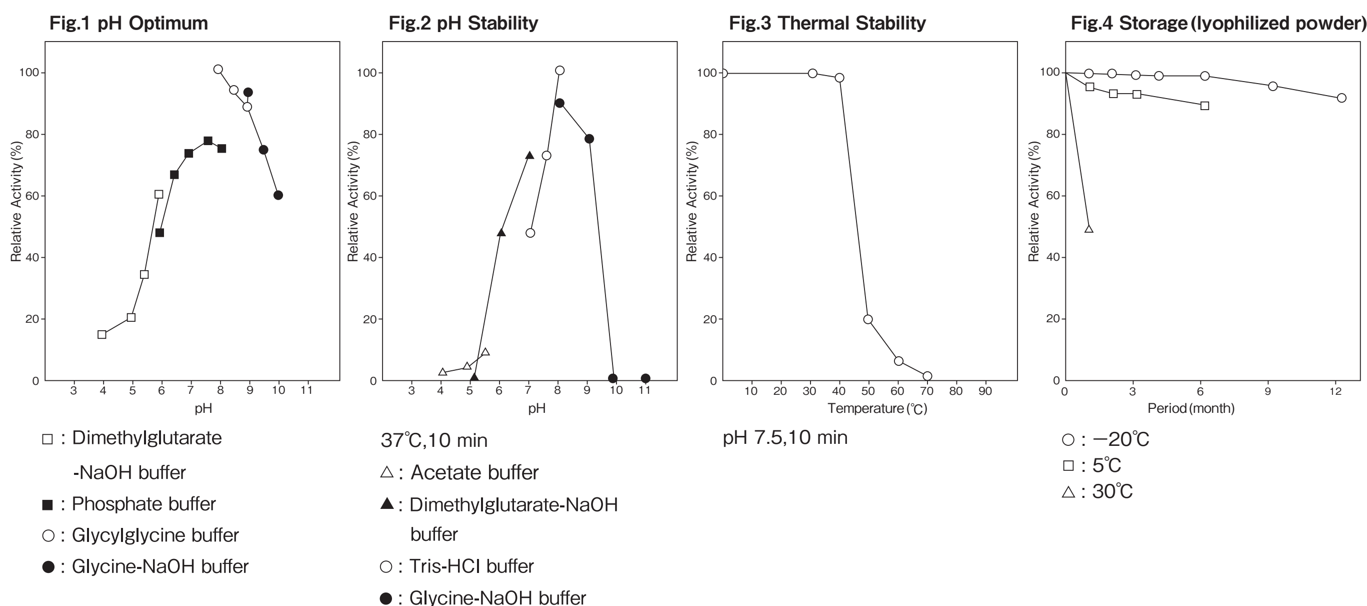


Table 1. Substrate specificity

Substrate	Relative activity (%)
Choline	100
Betaine aldehyde	46
Diethanolamine	1
Triethanolamine	3
N, N-Dimethylaminoethanol	5
N-Methylethanolamine	0

Table 2. Effect of various chemicals on COD activity

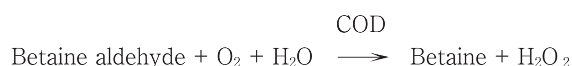
Additives	Concentration	Relative activity (%)
None	–	100
Triton X-100	0.1%	96
Adekatol SO-120	0.1%	106
Sodium laurylsulfate	0.1%	94
Deoxycholate	0.1%	94
Sodium laurylbenzene sulfate	0.1%	91
$CaCl_2$	5mM	101
$MgCl_2$	5mM	100
$FeCl_3$	5mM	0
$ZnCl_2$	5mM	8
$MnCl_2$	5mM	98
$CoCl_2$	5mM	31
$MoCl_2$	5mM	58
KCl	5mM	93
NaCl	5mM	97
NH_4Cl	5mM	100
LiCl	5mM	97
$BaCl_2$	5mM	103



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℃ 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℃). 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:
   FUJIFILM Wako Pure Chemical Corporation
   1st Grade #033-09812
   4-AA: NACALAI TESQUE, INC. Special grade #01907-52
   POD: Sigma Chemical Co. Type Ⅱ #P-8250
   EDTA (2 Na･$2H_2O$) : 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℃.
2. After 5 min, add 50 μl of enzyme solution and mix to start the reaction at 37℃.
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.

$$\triangle A/min \leqq 0.040\ Abs/min$$

■ Calculation

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

12.0 : millimolar extinction coefficient of quinoneimine dye at 500 nm ($cm^2/\mu mole$)

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℃ in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 4).

References

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3. Ohta-Fukuyama, M., Miyake, Y., Emi, S. and Yamano, T. (1989) J. Biochem., **88**, 197-203.
4. Takayama, M. et al. (1977) Clin. Chim. Acta, **79**, 93.
5. Sugawara, K. and Kihara, A. (1978) Eisei Kensa, **27** (1), 106-111.
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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℃）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：富士フイルム和光純薬製　一級 #033-09812

4-AA：ナカライテスク製　特級　#01907-52

POD：シグマ製　Type Ⅱ　#P-8250

EDTA（エチレンジアミン四酢酸・2Na・$2H_2O$）：キシダ化学製 #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℃で予備加温する。
2. 5 分経過後、酵素試料液 50 μl を正確に加えて混和し、37℃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。

$$\Delta A/min \leqq 0.040\ Abs/min$$

Ⅳ．計算

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

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

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 ：酵素試料液の検品濃度（mg/ml）