# CHOLINE OXIDASE [COD]

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

$$\text{Choline} + O_2 \longrightarrow \text{Betaine aldehyde} + H_2O_2$$
$$\text{Betaine aldehyde} + O_2 + H_2O \longrightarrow \text{Betaine} + H_2O_2$$



## 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 $1.2 \times 10^{-3}$M<br>Betaine aldehyde $8.7 \times 10^{-3}$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).

$$\text{Phosphatidylcholine} + H_2O \xrightarrow{\text{PLD}} \text{Choline} + \text{Phosphatidic acid}$$
$$\text{Choline} + 2\,O_2 + H_2O \xrightarrow{\textbf{COD}} \text{Betaine} + 2\,H_2O_2$$
$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

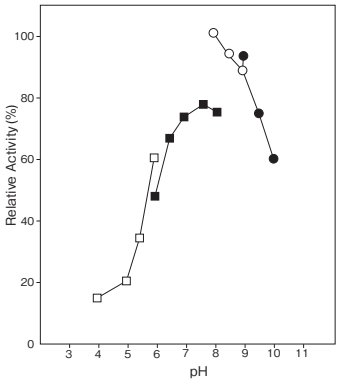
Table 1. Substrate specificity

| 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

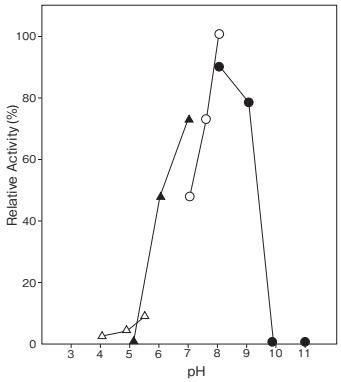
| Additives | Concentration | Relative activity (%) |
|---|---|---|
| None | – | 100 |
| Triton X-100 | 0.1% | 96 |
| Adekatol SO-120 | 0.1% | 106 |
| Sodium laurylsulfate | 0.1% | 94 |
| Deoxycholate | 0.1% | 94 |
| Sodium laurylbenzene sulfate | 0.1% | 91 |
| $CaCl_2$ | 5mM | 101 |
| $MgCl_2$ | 5mM | 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 |

**Fig.1 pH Optimum**



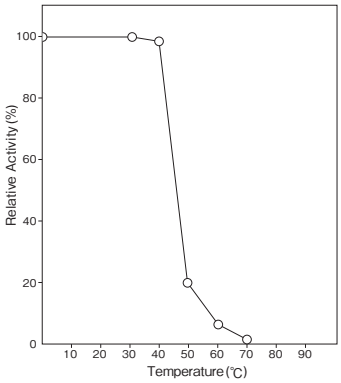

□ : Dimethylglutarate -NaOH buffer
■ : Phosphate buffer
○ : Glycylglycine buffer
● : Glycine-NaOH buffer

**Fig.2 pH Stability**



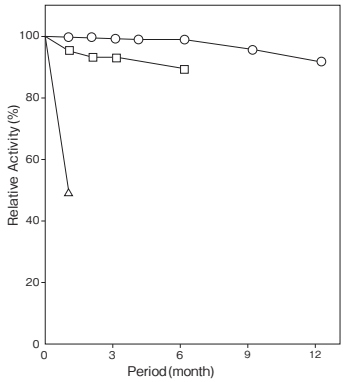

37℃,10 min
△ : Acetate buffer
▲ : Dimethylglutarate-NaOH buffer
○ : Tris-HCl buffer
● : Glycine-NaOH buffer

**Fig.3 Thermal Stability**




pH 7.5,10 min

**Fig.4 Storage (lyophilized powder)**




○ : −20℃
□ : 5℃
△ : 30℃

## 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:

$$\text{Choline} + O_2 \xrightarrow{\text{COD}} \text{Betaine aldehyde} + H_2O_2$$

$$\text{Betaine aldehyde} + O_2 + H_2O \xrightarrow{\text{COD}} \text{Betaine} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

### ■ 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


1. Ikuta, S., Matsuura, K., Imamura, S., Misaki, H. and Horiuchi, Y. (1977) J. Biochem., **82**, 157–163.
2. Ikuta, S., Imamura, S., Misaki, H. and Horiuchi, Y. (1977) ibid, **82**, 1741–1749.
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.
6. Okabe, H. et al. (1977) Clin. Chim. Acta, **80**, 87.


## 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）