Choline Oxidase (CHO)

C1492995

Storage Store at 2-8℃for short term (12 months). Store at -20℃for long term (24 months). Avoid freeze/thaw cycle.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction:

In enzymology, a choline oxidase (EC 1.1.3.17) is an enzyme that catalyzes the chemical reaction: choline +O_2↔ betaine aldehyde +H_2O_2. Thus, the two substrates of this enzyme are choline and O_2, whereas its two products are betaine aldehyde and H_2O_2. This enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with oxygen as acceptor.

$(CH_3)_3N^+CH_2CH_2OH + H_2O + 2O_2 \rightarrow (CH_3)_3N^+CH_2COO^- + 2H_2O_2$

PREPARATION and SPECIFICATION

Appearance: Yellowish amorphous powder, lyophilized.

Activity: ≥10 U/mg powder (containing approx. 20% of stabilizers).

Contaminant: Catalase ≤1.0 ×10² %.

Stabilizers: EDTA, BSA, amino acids (glycine, sodium glutamate, etc.).

PROPERTIES

Stability	Stable at -20℃ for at least one year	(Fig.1)
Molecular weight	approx. 95,000	
Isoelectric point	4.1±0.1	
Michaelis constants	2.84×10^{-3}M (Choline), 5.33×10^{-3}M(Betaine aldehyde)	
Structure	One mol of FAD is covalently bound to mol of the enzyme	
Inhibitors	p-Chloromercuribenzoate, Cu^{2+}, Co^{2+}, Hg^{2+}, Ag^+	
Optimum pH	8.0 - 8.5	(Fig.4)
Optimum temperature	40 - 45℃	(Fig.5)
pH Stability	pH 7.0 - 9.0 (30℃, 2 hr)	(Fig.6)
Thermal stability	below 37℃ (pH 7.5, 10min)	(Fig.7)
Effect of various chemicals		(Table.1)

APPLICATIONS

This enzyme is useful for enzymatic determination of phospholipids when coupled with phospholipase D and for choline esterase-activity in clinical analysis.

ASSAY

Principle:

$$\text{Choline} + H_2O + 2O_2 \xrightarrow{\text{choline oxidase}} \text{Betaine} + 2H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

The appearance of quinoneimine dye is measured at 500nm by spectrophotometry.

Unit definition:

One unit causes the formation of one micromole of hydrogen peroxide (half a micromole of quinoneimine dye) per minute under the conditions described below.

Method: Reagents

A. Choline chloride solution: 2.1% [2.1g choline chloride/100ml of Tris-HCl buffer (D)] (Should be prepared fresh)

B. 4-AA solution: 1.0% (1.0g 4-aminoantipyrine/100ml of H_2O) (Store at 4℃ in a brownish bottle)

C. Phenol solution: 1.0% (1.0g phenol/100ml of H_2O) (Store at 4℃ in a brownish bottle)

D. Tris-HCl buffer: 0.1M Tris-HCl buffer, pH 8.0. Dissolve 12.1g of Tris (MW=121.14) in ca.800ml of H_2O and, after adjusting the pH to 8.0 at 25℃ with 2.0 N HCl, fill up to 1,000ml with H_2O.

E. Enzyme diluent: 10mM Tris-HCl buffer, pH 8.0 contg. 2mM EDTA and 1.0% KCl.

Procedure

1. Prepare the following working solution (100ml) in a brownish bottle before use and store on ice in a brownish bottle.

97 ml	Substrate solution	(A)
1.0ml	4-AA solution	(B)
2.0ml	Phenol solution	(C)
5.0mg	Peroxidase from horseradish (110 purpurogallin units/mg)	

Concentration in assay mixture

Tris buffer	97 mM
Choline chloride	0.14 μM
EDTA	33 mM
KCl	2.2 mM

4-Aminoantipyrine	0.48 mM
Phenol	2.1 mM
POD	ca.4.92U/ml

2. Pipette 3.0ml of working solution into a cuvette (d 1.0cm) and equilibrate at 37℃ for about 5 minutes.
3. Add 0.05ml of the enzyme solution* and mix by gentle inversion.
4. Record the increase in optical density at 500nm against the working solution for 3 to 4 minutes in a spectrophotometer thermostated at 37℃, and calculate the ΔOD per minute from the initial linear portion of the curve.

*Dissolve the enzyme preparation in ice-cold Tris-HCl buffer (D) and dilute to 0.1−0.5U/ml with enzyme diluent (E).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min} \times \text{Vt} \times \text{df}}{12.0 \times 1/2 \times 1.0 \times \text{Vs}} = \Delta\text{OD/min} \times 10.17 \times \text{df}$$

Vt: Total volume (3.05ml)

Vs: Sample volume (0.05ml)

12.0: Millimolar extinction coefficient of quinoneimine dye under the assay conditions (cm^2/micromole)

1/2: Factor based on the fact that one mole of H_2O_2 produces half a mole of quinoneimine dye.

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration in dissolution (c mg/ml)

Table 1. Effect of Various Chemicals on Choline oxidase

[The enzyme dissolved in 10mM Tris-HCl buffer, pH 8.0 contg. 2mM EDTA and 1.0% KCl (5U/ml) was incubated with each chemical at 25℃ for 1hr.]

Chemical	Concn(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	MIA	2.0	87
$MgCl_2$	2.0	87	NEM	2.0	100
$CaCl_2$	2.0	92	IAA	2.0	95
$Ba(OAc)_2$	2.0	89	Hydroxylamine	2.0	77
$FeCl_3$	2.0	87	EDTA	5.0	92
$CoCl_2$	2.0	89	o-Phenanthroline	2.0	90
$MnCl_2$	2.0	91	α,α′-Dipyridyl	1.0	91
$ZnCl_2$	2.0	88	Borate	50	94
$CdCl_2$	2.0	92	NaF	2.0	92
$NiCl_2$	2.0	91	NaN_3	2.0	92
$CuSO_4$	2.0	92	Triton X-100	0.10%	96
$Pb(OAc)_2$	2.0	87	Brij 35	0.10%	92
$AgNO_3$	2.0	80	Tween 20	0.10%	95
$HgCl_2$	2.0	48	Span 20	0.10%	94
2-Mercaptoethanol	2.0	90	Na-cholate	0.10%	96
PCMB	1.0	13	SDS	0.05%	95
			DAC	0.05%	91

Ac, CH_3CO; PMB, p-chloromercuribenzoate; MA, Moniodoacetate; NEM, N-Ethylmaleimide; IAA, Iodoacetamide; EDTA, Ethylenediaminetetraacetate; SDS, Sodium dodecyl sulfate; DAC, Dimethylbenzylalkylammonium chloride.

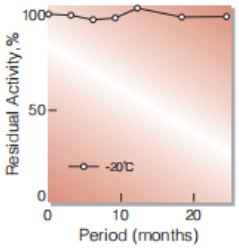

Fig.1. Stability (Powder form)

(kept under dry conditions)

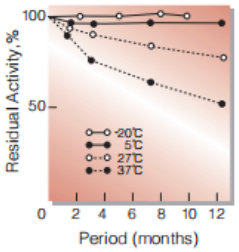

Fig.2. Stability (Powder form)

(kept under dry conditions)

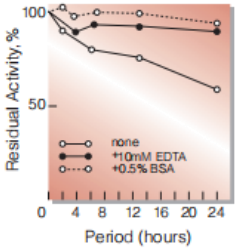

Fig.3. Stability (Liquid form at 37℃)

(enzyme concentration: 1.0mg/ml
buffer composition: 0.1M K-phosphate buffer, pH7.5)

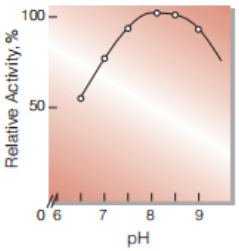

Fig.4. pH-Activity

(37℃, in 50mM K-phosphate buffer)

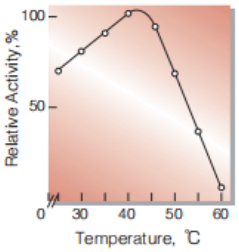

Fig.5. Temperature activity

(in 50mM K-phosphate buffer, pH7.5)

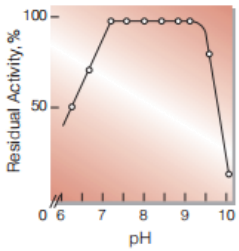

Fig.6. pH-Stability

(30℃,2hr-treatment with 50mM buffer solution:pH6.0-9.0, K-phosphate; pH9.0-10.0, glycine-NaCl-NaOH.)

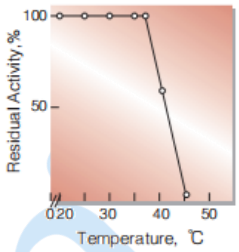

Fig.7. Thermal stability

(15min-treatment with 50mM K-phosphate buffer, pH7.5)