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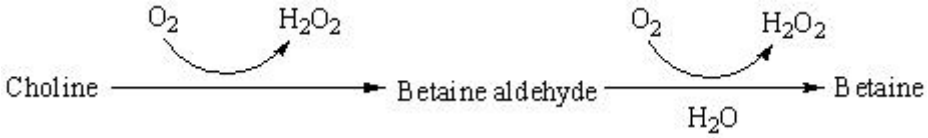
TECHNICAL INFORMATION

Catalog Number: 153492
Choline oxidase

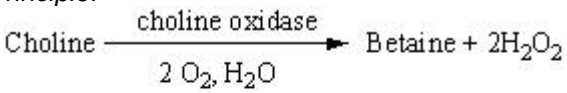
Molecular Weight: Approximately 95,000
CAS # : 9028-67-5
Synonym: Choline (Betaine aldehyde) : Oxigen oxidoreductase
E.C. 1.1.3.17
Physical Appearance: Yellowish powder
Source: *Alcaligenes sp.*
Principle:

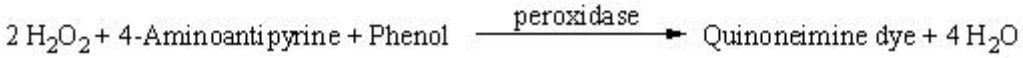


Activity: Approximately 10 u/mg solid
Typical Contaminant: Catalase - Not more than 1.0 × 10^{2}%
Stabilizers: (contains approximately 20% stabilizers) EDTA, bovine serum albumin, amino acids (glycine, sodium glutamate, etc.)
Isoelectric Point: 4.1 ± 0.1
Michaelis Constants:

2.84 × 10^{-3} M (Choline)
5.33 × 10^{-3} M (Betaine aldehyde)

Composition: One mole of FAD is covalently bound to one mole of the enzyme.[8]
Inhibitors: p-Chloromercuribenzoate, Cu^{++}, Co^{++}, Hg^{++}, Ag^{+}
Optimum pH: 8.0 - 8.5
Optimum Temperature: 40-45°C
pH Stability: pH 7.0 - 9.0 (30°C, 2 hours)
Thermal Stability: Below 37°C (pH 7.5, 10 minutes)
Use: The enzyme is useful for enzymatic determination of phospholipids by coupling with phospholipase D and for cholinesterase activity assays.[9-11]
Activity Assay:
Principle:





The appearance of quinoneimine dye is measured at 500 nm 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.
Reagents:

A. Choline chloride solution: 2.1% [2.1 g choline chloride/100 ml of Tris buffer D. Prepare Fresh for each use].
B. 4-AA solution: 1.0% [1.0 g 4-Aminoantipyrine/100 ml of water. Store at 4°C in a dark bottle]
C. Phenol Solution: 1.0% [1.0 g phenol/100 ml of water. Store at 4°C in a dark bottle]
D. Tris Buffer: 0.1 M Tris-HCl buffer, pH 8.0 [Dissolve 12.1 g of Tris in approximately 800 ml of water and, after adjusting the pH to 8.0 at 25°C with 2.0 N HCl, fill up to 1000 ml with water]
E. Enzyme Diluent: 10 mM Tris-HCl buffer, pH 8.0 containing 2 mM EDTA and 1.0% KCl.

Procedure:

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

97 ml	Substrate solution	(Reagent A)
1.0 ml	4-AA Solution	(Reagent B)
2.0 ml	Phenol Solution	(Reagent C)
5.0 mg	Peroxidase from horseradish (100 purpurogallin units/mg)	

Concentration in Assay Mixture will be:

Tris Buffer	97 mM
Choline Chloride	0.14 M
EDTA	33 uM
KCl	2.2 mM
4-Aminoantipyrine	0.48 mM
Phenol	2.1 mM
POD	approximately 0.98 u/ml

2. Pipette 3.0 ml of working solution into a cuvette (d = 1.0 cm) and equilibrate at 37°C for about 5 minutes.
3. Add 0.05 ml of the enzyme solution* and mix by gentle inversion.
4. Record the increase of optical density at 500 nm against the working solution for 3 to 4 minutes in a spectrophotometer thermostated at 37°C, and calculate the DOD per minute from the initial linear portion of the curve.
* Dissolve the enzyme preparation in ice-cold Tris buffer (Reagent D) and dilute to 0.1 to 0.5 units/ml with enzyme diluent (Reagent E).
Calculation:
Activity can be calculated by using the following formula:

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

$$= \Delta OD/min \times 10.17 \times df$$

$$\text{Weight activity (U/mg)} = (U/ml) \times 1/C$$

Where:
Vt: Total volume (3.05 ml)
Vs: Sample volume (0.05 ml)
12.0: Millimolar extinction coefficient of quinoneimine dye under the assay condition (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)
References:

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