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Product Information

Cholesterol Oxidase from microorganisms

Catalog Number **C8868**
Storage Temperature –20 °C

CAS RN 9028-76-6
EC 1.1.3.6
Synonyms: Cholesterol:oxygen oxidoreductase; β-hydroxy steroid oxidoreductase; CHOD; 3β-hydroxysteroid:oxygen oxidoreductase

Product Description
Cholesterol oxidase (CHOD) catalyzes the first step in cholesterol catabolism. Some non-pathogenic bacteria, such as *Streptomyces* are able to utilize cholesterol as a carbon source. Pathogenic bacteria, such as *Rhodococcus equi*, require CHOD to infect a host's macrophage.[1]

CHOD is bifunctional. Cholesterol is initially oxidized to cholest-5-en-3-one in an FAD-requiring step. The cholest-5-en-3-one is isomerized to cholest-4-en-3-one.[1] The isomerization reaction may be partially reversible.[2] The activity of CHOD depends on the physical properties of membrane to which the substrate is bound.[3] The net reaction is:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{cholest-4-en-3-one} + H_2O_2$$

CHOD is used to determine serum cholesterol.[4,5] It is the second most widely used enzyme in diagnostic applications after glucose oxidase.[6] CHOD also finds application in the microanalysis of steroids in food samples and in distinguishing 3-ketosteroids from 3β-hydroxysteroids.[7]

Transgenic plants expressing cholesterol oxidase are being investigated in the fight against the cotton boll weevil.[8] Cholesterol oxidase has also been used as a molecular probe to elucidate cellular membrane structures [3,9]

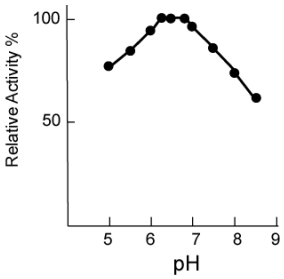
Cholesterol oxidase is a monomeric flavoprotein containing FAD.[1]
Molecular mass:[10] 64 kDa
Cofactor:[11] FAD

Inhibitors:[12] $AgNO_3$, $HgCl_2$, 0.1% SDS

Isoelectric point (pI):[10] 4.7

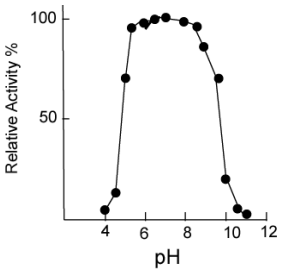
pH optimum:[10] 6.5 (see Figure 1)

Figure 1.
pH Profile of Enzyme Activity



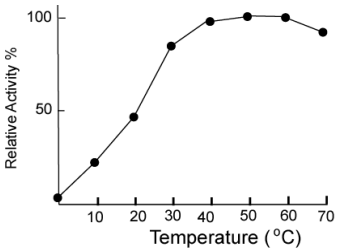
pH range:[10] Maximal activity retained from pH 5.3–7.5 (see Figure 2)

Figure 2.
pH Profile of Enzyme Activity



Temperature optimum:[10] 50 °C (see Figure 3)

Figure 3.
Temperature Profile of Enzyme Activity



This product is supplied as a lyophilized powder containing ≥15% protein with sucrose.

Specific activity: ≥50 units/mg protein

Unit definition: one unit will convert 1.0 µmole of cholesterol to 4-cholesten-3-one per minute at pH 7.5 at 25 °C.
Note: 4-cholesten-3-one may undergo isomerization.

CHOD is assayed spectrophotometrically in a 3.0 ml reaction mixture containing 38 mM potassium phosphate, 0.009% (w/v) *o*-dianisidine, 0.017% (w/v) cholesterol, 0.33% (v/v) Triton™ X-100, 10 units of peroxidase, and 0.01–0.02 unit of cholesterol oxidase.

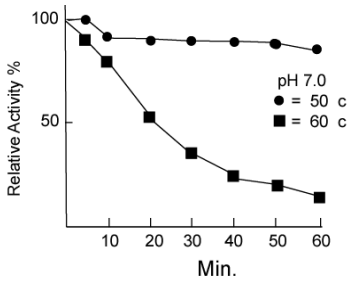
Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
CHOD is soluble in cold 50 mM potassium phosphate buffer, pH 7.5. Prepare solutions immediately before use.

Storage/Stability
Store product at –20 °C with desiccation. When stored at –20 °C, the enzyme retains activity for at least 9 months.

At 50 °C, no loss of activity is observed after 5 minutes; 15% loss of activity is observed after 60 minutes.[10] (see Figure 4)

Figure 4.
Temperature-dependent activity versus time



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