Product Information

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Cholesterol Oxidase
from *Streptomyces* sp.

Catalog Number **C8649**
Storage Temperature –20 °C

CAS RN 9028-76-6
EC 1.1.3.6
Synonyms: Cholesterol:oxygen oxidoreductase; 3β-hydroxy steroid oxidoreductase; CHOD; 3β-hydroxysteroid:oxygen oxidoreductase; cholesterol-O_2 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:

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

Typically cholesterol oxidase is isolated from Gram-positive bacteria. CHOD from *Streptomyces*, *Cellulomonas*, and *Brevibacterium* have been found to be essentially equivalent analytically.[4]

CHOD is used to determine serum cholesterol.[4,5] It is the second most widely used enzyme in clinical 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] 50 kDa (SDS-PAGE)
Cofactor:[10] FAD

pH Optimum:[10] 6.0

pH Range:[10] 6.0–8.0

Temperature optimum:[10] 60 °C

Substrates:[7]

cholesterol	estrone
cholest-5-en-3β-ol-7-one	dihydrocholesterol
dehydroisoandrosterone	pregnenolone

K_M (μM):

Cholesterol[10]	13.0
Pregnenolone[7]	0.023
Dehydroepiandrosterone[11]	0.0275

Inhibitors:
Fenpropimorph:[6] 50 mg/l, 50% inhibition
Sarkosyl:[12] 1%, 56% inhibition

This product is purified from *Streptomyces* sp. and is supplied as a lyophilized powder containing ~60% protein (biuret), BSA, sodium cholate, and borate.

Specific activity: ≥20 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 7.0. Prepare solutions immediately before use.

Storage/Stability
The product ships on dry ice and storage at –20 °C with desiccation is recommended. When stored at –20 °C, the product retains activity for at least two years.

A solution of CHOD in 50 mM potassium phosphate at pH 7.0 can lose 50% of its activity in 15 minutes at 60 °C.[3]

References

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