

# Product Information

sigma-aldrich.com

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 microbial, recombinant expressed in *Escherichia coli*

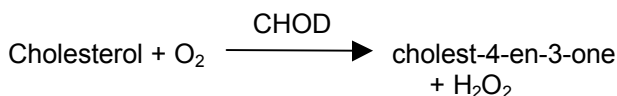
Catalog Number **C1235**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

CAS RN 9028-76-6  
EC 1.1.3.6  
Synonyms: Cholesterol:oxygen oxidoreductase;  
 $3\beta$ -hydroxy steroid oxidoreductase; CHOD;  
 $3\beta$ -hydroxysteroid:oxygen oxidoreductase;  
cholesterol- $\text{O}_2$  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.<sup>1</sup>

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.<sup>1</sup> The isomerization reaction may be partially reversible.<sup>2</sup> The activity of CHOD depends on the physical properties of membrane to which the substrate is bound.<sup>3</sup> The net reaction is:



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

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

Cholesterol oxidase is a monomeric flavoprotein containing FAD.<sup>1</sup>

Molecular mass: 55 kDa (SDS-PAGE)  
Cofactor: FAD

pH Optimum: 7.0

pH Stability: 5.0–0.0

$K_M$ :  $3.5 \times 10^{-4}$  M (cholesterol)

Inhibitors:  $\text{Hg}^{2+}$ ,  $\text{Ag}^+$ , ionic detergents

This product is a recombinant, microbial enzyme purified from *E. coli*. It is supplied as a lyophilized powder containing ~25% protein (biuret), phosphate buffer salts, and EDTA.

Specific activity:  $\geq 50$  units/mg protein

Unit definition: one unit will convert 1.0  $\mu\text{mole}$  of cholesterol to 4-cholesten-3-one per minute at pH 7.5 at  $25\text{ }^{\circ}\text{C}$ .

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<sup>®</sup> X-100, 10 units of peroxidase, and 0.01–0.02 unit of cholesterol oxidase.

Other activities:

Catalase: none detected (detection limit, 1.0% of cholesterol oxidase activity)

Cholesterol esterase: none detected (detection limit, 0.01% of cholesterol oxidase activity)

### 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

Store product at  $-20^{\circ}\text{C}$  with desiccation. When stored at  $-20^{\circ}\text{C}$ , the enzyme retains activity for at least one year.

The activity of the enzyme in solution can decrease rapidly above  $55^{\circ}\text{C}$ .

### References

1. Caldinelli, L., *et al.*, Dissecting the structural determinants of the stability of cholesterol oxidase containing covalently bound flavin. *J. Biol. Chem.*, **280**, 22572-81 (2005).
2. Smith, A.G., and Brooks, C.J.W., The mechanism of the isomerization of cholest-5-en-3-one to cholest-4-en-3-one by cholesterol oxidase. *Biochem. Soc. Trans.*, **5**, 1088-90 (1977).
3. Ahn, K-W, and Sampson, N.S., Cholesterol oxidase senses subtle changes in lipid bilayer structure. *Biochemistry*, **43**, 827-36 (2004).
4. Allain, C.C., *et al.*, Enzymatic determination of total serum cholesterol. *Clin. Chem.*, **20**, 470-75 (1974).
5. Lolekha, P.H., *et al.*, Performance of four sources of cholesterol oxidase for serum cholesterol determination by the enzymatic endpoint method. *Clin. Chim. Acta*, **339**, 135-45 (2004).
6. MacLachlan, J., *et al.*, Cholesterol oxidase: Sources, physical properties and analytical applications. *J. Steroid Biochem. Mol. Biol.*, **72**, 169-95 (2000).
7. Toyama, M., *et al.*, Alteration of substrate specificity of cholesterol oxidase from *Streptomyces* sp. by site-directed mutagenesis. *Protein Eng.*, **15**, 177-84 (2002).
8. Corbin, D.R., *et al.*, Expression and chloroplast targeting of cholesterol oxidase in transgenic tobacco plants. *Plant Physiology*, **126**, 1116-28 (2001).
9. Pal, R., *et al.*, Effect of cholesterol concentration on organization of viral and vesicle membranes. *J. Biol. Chem.*, **255**, 5802-06 (1980).

TRITON is a registered trademark of Union Carbide Corp.

SKM,RBG,JWM,MAM 11/07-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.