

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 °C

CAS RN 9028-76-6

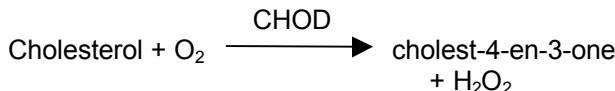
EC 1.1.3.6

Synonyms: Cholesterol:oxygen oxidoreductase; 3 β -hydroxy steroid oxidoreductase; CHOD; 3 β -hydroxysteroid:oxygen oxidoreductase; cholesterol-O₂ 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:



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 3 β -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: 55 kDa (SDS-PAGE)

Cofactor: FAD

pH Optimum: 7.0

pH Stability: 5.0–0.0

K_M: 3.5 × 10^{–4} M (cholesterol)

Inhibitors: Hg²⁺, 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: ≥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) σ -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.

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 °C with desiccation. When stored at -20 °C, the enzyme retains activity for at least one year.

The activity of the enzyme in solution can decrease rapidly above 55 °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.