

Product Information

Alcohol Oxidase from *Hansenula polymorpha*

Product Number **A0438**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

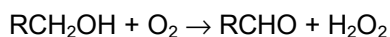
CAS RN 9073-63-6

EC 1.1.3.13

Synonym: Alcohol: oxygen oxidoreductase

Product Description

Alcohol oxidase catalyzes the oxidation of short-chain, primary, aliphatic alcohols to the respective aldehydes.



The enzyme has the highest affinity for methanol with the affinity decreasing with increasing chain length of the alkyl (R) group.

Alcohol oxidase plays a major role in the metabolism of methanol resulting in the formation of formaldehyde and has been detected in several genera of yeasts, such as *Candida*, *Pichia*, and *Hansenula*, that utilize methanol as a sole carbon and energy source.^{1,2}

Primarily localized in the peroxisome, alcohol oxidase has also been found in the cytoplasm. Monomers are synthesized in the cytosol and assembled into octomers in the peroxisome. Octomerization is thought to be chaparone mediated.³ Alcohol oxidase is of interest for the study of protein translocation into peroxisomes.⁴

K_M (mM):^{4,5}

methanol	2.15
benzyl alcohol	26.6
ethanol	16.2
formaldehyde	2.6
allyl alcohol	33.3
n-butanol	166
n-propanol	66

Substrates (relative reaction rate):⁵

methanol	1.0
ethanol	0.92
benzyl alcohol	0.81
n-propanol	0.72
n-butanol	0.57
ethylene glycol	0.09
isopropanol	0.07
isoamyl alcohol	0.02

also 2-propene-1-ol, allyl alcohol, formaldehyde

Molecular Weight:⁶ 670 kDa (octomer, gel filtration)

Alcohol oxidase is a homooctomeric flavoprotein with eight equal subunits of approximately 83 kDa; each of which contains a flavin adenine dinucleotide (FAD) molecule.⁶

Cofactor:⁴ FAD, one molecule/subunit

Isoelectric point:⁷ 6.1

pH Range:⁸ 6.7–9.8

pH Optimum:⁸ 8.5

K_i (mM):⁵

methanol 6,500

Inhibitors:^{3,8}

1,4-butyne diol (irreversible)
 4-hydroxy-2-butanal
 cyclopropanol
 cyclopropanone (suicide substrate)
 formaldehyde, H_2O_2 (5–10 mM)
 hydroxylamine, KBr, KCN
 sodium azide, urea

This product (A0438) is purified from *Hansenula polymorpha* and is supplied as an orange vacuum-dried powder.

Protein: 30–60% (Bradford), balance primarily buffer salts.

Specific activity: 20–40 units/mg protein.

Unit definition: One unit will oxidize 1.0 μmole of methanol to formaldehyde per minute at pH 7.5 at $25\text{ }^{\circ}\text{C}$.

Alcohol oxidase is assayed spectrophotometrically in a 3.02 ml reaction mixture containing 96 mM potassium phosphate, pH 7.5, 0.033% (v/v) methanol, 2 mM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), 0.00001% (w/w) hydrogen peroxide, 2.5 units peroxidase, and 0.01 unit alcohol 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

Alcohol oxidase is soluble (0.1 unit/ml) in cold 100 mM potassium phosphate, pH 7.5 at 25 °C. Solutions should be prepared just before use.

Storage/Stability

Store the product, as supplied, at –20 °C. It is stable for at least 2 years when stored at –20 °C.

References

1. Kato, N., *et al.*, *Agric. Biol. Chem.*, **38**, 675 (1974).
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3. Evers, M.E., *et al.*, Assembly of alcohol oxidase in peroxisomes of the yeast *Hansenula polymorpha* requires the cofactor flavin adenine dinucleotide. *Mol. Biol. Cell*, **5**, 829-37 (1994).
4. van der Klei, I.J., *et al.*, Alcohol oxidase from *Hansenula polymorpha* CBS 4732. *Methods Enzymol.*, **188**, 420-27(1990).
5. Badea, M., and Arsene, M.L., Catalytic properties of alcohol oxidase to oxidize aliphatic and aromatic alcohols. *Prog. Catal.*, **5**, 45-60 (1996).
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7. Shleev, S.V., *et al.*, Purification and characterization of alcohol oxidase of a genetically constructed over-producing strain of the methylotropic yeast *Hansenula polyforma*. *Biochemistry (Mosc.)*, **71**, 245-50 (2006).
8. Couderc, R., and Baratti, J., Oxidation of methanol by the yeast *Pichia pastoris*: purification and properties of alcohol oxidase. *Agric. Biol. Chem.*, **44**, 2279-89 (1980).
9. Bystryck, L.V., *et al.*, Modification of flavin adenine dinucleotide in alcohol oxidase of the yeast *Hansenula polymorpha*. *J. Gen. Microbiol.*, **137**, 2381-86 (1991).

VLR,JWM,MAM 11/06-1

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