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ProductInformation

Alcohol Oxidase
from *Candida boidinii*

Catalog Number **A6941**
Storage Temperature –20 °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.

$$RCH_2OH + O_2 \rightarrow RCHO + H_2O_2$$

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):[5]

Methanol	3
2-Propin-1-ol	10
1,4-Butynediol	36
4-Hydroxy-2-butynal	0.44

Other substrates:[2,5]

methanol	ethanol
n-propanol	n-butanol
chloroethanol	2-propenol-1-ol
2-propynol-1-ol	2-butenol-1-ol
1,4-butynoldiol	formaldehyde

The enzyme shows little activity toward secondary, tertiary, or aromatic alcohols; or aliphatic alcohols with a chain length of more than 5 carbons.[2,6,7]

Molecular Mass:[5] 600 kDa (octomer, sedimentation equilibrium)

Alcohol oxidase is a homooctomeric flavoprotein with eight equal 74 kDa subunits; each containing a flavin adenine dinucleotide (FAD) molecule.[2]

Cofactor:[2] FAD, one molecule/subunit

Isoelectric point:[8] 5.4–5.8

pH Range:[9] 6.5–8.5

pH Optimum:[2] 7.5

Inhibitors:[5,6,9]

H_2O_2	propynal
PCMB	iodoacetate
1,4-butynediol	sodium acetate
cyclopropanone	4-hydroxy-2-butynal
diethyldicarbonate	

Hg^{2+} ion (reversible by 2-mercaptoethanol)
Ag^{+} and Cu^{2+} ions (95% inhibition at 1 mM, EDTA reversible)

This product (A6941) is purified from *Candida boidinii* and is supplied as a lyophilized powder containing potassium phosphate buffer salts, DTE, and stabilizer.

Protein content: ~10% (biuret)

Specific activity: 5-15 units/mg protein

Unit definition: One unit will oxidize 1.0 µmole of methanol to formaldehyde per minute at pH 7.5 at 25 °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 (1 mg protein/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).
2. Sahm, H., *et al.*, Alcohol oxidase from *Candida boidinii*. Methods Enzymol., **89,** 424-28 (1982).
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. Nichols, C.S., and Cromartie, T.H., Irreversible inactivation of the flavoenzyme alcohol oxidase with acetylenic alcohols. Biochem. Biophys. Res. Commun., **97**, 425-42 (1980).
6. Cromartie, T.H., *Biochem. Biophys. Res. Commun*., **105**, 785-790 (1982).
7. Aggelis, G., *et al*., *J. Biotechnol*. **72**, 127-139 (1999).
8. Bringer, S., et al., Purification and properties of alcohol oxidase from Poria contigua. Eur. J. Biochem., **101,** 563-70 (1979).
9. Cromartie, T.H., Sulfhydryl and histidinyl residues in the flavoenzyme alcohol oxidase from *Candida boidinii*. Biochemistry, **20**, 5416-23 (1981).

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