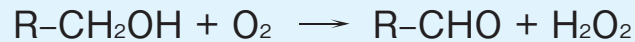


ALCOHOL OXIDASE [ALOD]

from *Candida* sp.

(Alcohol: oxygen oxidoreductase, EC 1.1.3.13)



Preparation and Specification

Appearance : Light yellowish amorphous powder, lyophilized
 Specific activity : More than 7 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 520 kDa (gel filtration) 75 kDa (SDS-PAGE)	
Isoelectric point	: pH 4.1	
Michaelis constants	: Methanol $2.9 \times 10^{-3}\text{M}$ Ethanol $8.2 \times 10^{-3}\text{M}$	
Optimum pH	: 7.5–9.0	Figure 1
pH stability	: 6.0–9.5 (37°C, 60 min)	Figure 2
Thermal stability	: Stable at 40°C and below (pH 7.5, 10 min)	Figure 3
Effect of chemicals	: See Table 2 and Table 3	
Inhibitors	: PCMB, N-Ethyl maleinimide	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **blood alcohol**.

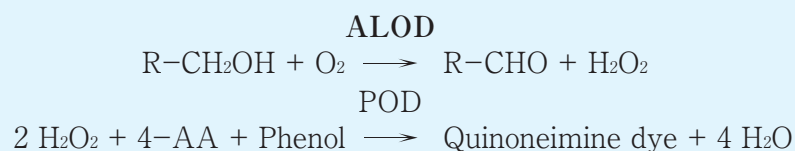


Table 1. Substrate specificity

Substrate	Relative activity (%)
Methanol	100
Ethanol	79.3
n-Propanol	46.5
iso-Propanol	25.8
n-Butanol	39.6
Form aldehyde	48.2
Acetoaldehyde	0

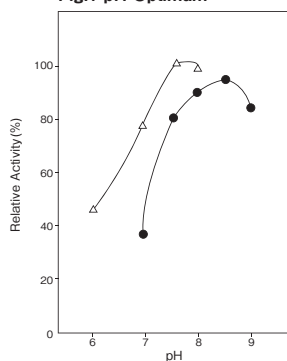
Table 3. Effect of detergents on ALOD activity

Detergent	Concentration (%)	Relative activity (%)
Nonidet P-40	0.1	96.3
Triton X-100	0.1	98.7
Adekamol PC-8	0.1	91.5
Adekamol SO-120	0.1	96.3
Tween 80	0.1	98.7
Brij 35	0.1	95.1
Deoxycholate	0.1	69.8

Table 2. Effect of metal ions on ALOD activity

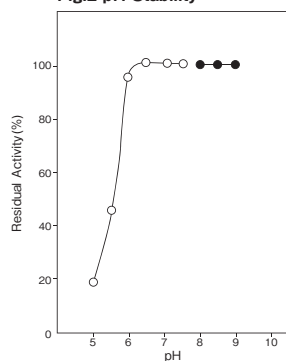
Metal ion	Concentration (mM)	Relative activity (%)
None	-	100.0
KCl	10	103.4
NaCl	10	99.3
LiCl	10	99.3
NH ₄ Cl	10	95.2
MgCl ₂	1	100.0
CaCl ₂	1	93.1
CoCl ₂	1	68.4
BaCl ₂	1	69.8
NiCl ₂	1	70.5
MnCl ₂	1	100.6
EDTA	1	108.2

Fig.1 pH Optimum



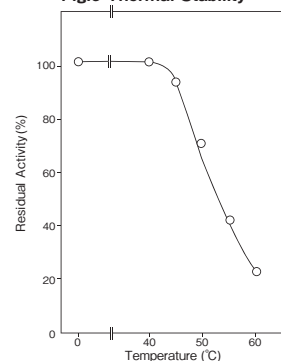
△ : Phosphate buffer
● : Tris-HCl buffer

Fig.2 pH Stability



(+0.2M NaCl) 37°C, 60 min.
○ : 3,3-Dimethylglutarate-NaOH buffer
● : Tris-HCl buffer

Fig.3 Thermal Stability

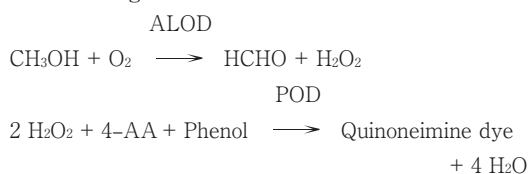


pH 7.5, 10 min.
10 mM Tris-HCl buffer

Assay

Principle

The assay is based on the increase in absorbance at 480 nm as the formation of quinoneimine dye proceeds in the following reactions:



Unit definition

One unit is defined as the amount of enzyme which generates 1 μmole of H_2O_2 and formaldehyde from methanol per minute at 37°C under the conditions specified in the assay procedure.

Reagents

- Reaction mixture

0.2 M Tris-HCl buffer	pH8.0	0.20 ml
15 mM 4-AA solution		0.10 ml
0.2% (W/V) Phenol solution		0.10 ml
2.0 M Methanol solution		0.25 ml

50 U/ml POD solution ¹⁾	0.10 ml
Distilled water	0.25 ml

1): 50 U/ml POD solution

Dissolve 500 U (PPU) of POD with 10 ml of distilled water.

2. Reaction stopper

Ethanol

3. Enzyme dilution buffer

10 mM Tris-HCl buffer pH 8.0

4. Reagents

4-AA: NACALAI TESQUE, INC. Special grade #01907-52

POD: Sigma Chemical Co. Type II #P-8250

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 1.0 ml of reaction mixture into a small test tube and preincubate at 37°C.

2. After 5 min, add exactly 50 μl of enzyme solution and mix to start the reaction at 37°C.

※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.

3. At 5 min after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.

4. Measure the absorbance at 480 nm.

Absorbance sample : As
blank : Ab

$$\Delta A = (As - Ab) \leq 0.20 \text{ Abs}$$

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A/5}{17.17 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

17.17 : millimolar extinction coefficient of quinoneimine dye at 480 nm (cm² / μmole)

1/2 : a multiplier derived from the fact that 2 mole of H₂O₂ produces 1 mole of quinoneimine dye

5 : reaction time (min)

3.05 : final volume (ml)

0.05 : volume of enzyme solution (ml)

X : concentration of the sample in enzyme solution

(mg/ml)

Storage

Storage at -20°C in the presence of a desiccant is recommended.

References

1. Fujii, T. and Tonomura, K. (1972) Agric. Biol. Chem., **36**, 2297-2306.
2. Sahm, H. and Wagner, F. (1973) Eur. J. Biochem., **36**, 250-256.
3. Kato, N., Omori, Y., Tani, Y. and Ogata, K. (1976) Eur. J. Biochem., **64**, 341-350.
4. Tani, Y., Miya, T., Nishikawa, H. and Ogata, K. (1972) Agric. Biol. Chem., **36**, 68-75.

ALOD 活性測定法 (Japanese)

I. 試薬液

1. 反応試薬混合液

0.2M トリス-HCl 緩衝液 pH8.0	0.20 ml
15mM 4-AA 溶液	0.10 ml
0.2% (W/V) フェノール溶液	0.10 ml
2M メタノール液	0.25 ml
50U/ml POD 溶液 ¹⁾	0.10 ml
精製水	0.25 ml

1): 50U/ml POD 溶液

POD 500 単位 (PPU) を精製水 10ml で溶解する。

2. 反応停止液

エタノール原液をそのまま使用する。

3. 酵素溶解希釈用液

10mM トリス-HCl 緩衝液 pH8.0

4. 試薬

4-AA: ナカライテスク社製 特級 #01907-52

POD: シグマ社製 Type II #P-8250

II. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。

その液を酵素溶解希釈用液で適宜希釈する。

III. 測定操作法

1. 小試験管に反応試薬混合液 1.0ml を正確に分注して 37°C で予備加温する。

2. 5 分経過後、酵素試料液 50 μl を正確に加えて混和後、37°C で反応を開始する。

※ 盲検は酵素試料液の代わりに酵素溶解希釈用液 50 μl を加える。

3. 5 分経過後、反応停止液 2.0ml を正確に加え反応を停止する。

4. 480nm における吸光度を測定する。

求められた吸光度を試料液は As、盲検液は Ab とする。

$$\Delta A = (As - Ab) \leq 0.20 \text{ Abs}$$

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/5}{17.17 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

17.17: キノンイミン色素の 480nm におけるミリモル分子吸光数 (cm²/ μmole)

1/2: H₂O₂ 2 モルからキノンイミン色素 1 モルが生成することによる係数

5 : 反応時間 (min)

3.05 : 反応総液量 (ml)

0.05 : 反応に供した酵素試料液量 (ml)

X : 酵素試料液中の検品濃度 (mg/ml)