



乙醇氧化酶

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 $2.9 \times 10^{-3}M$

Ethanol $8.2 \times 10^{-3}M$

Optimum pH : 7.5–9.0 Figure 1

pH stability : 6.0–9.5 (37°C, 60 min) Figure 2

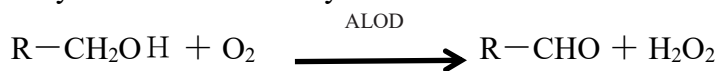
Thermal stability : Stable at 40°C and below
(pH 7.5, 10 min) Figure 3

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:





上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话: 400-666-5481 传真: 021-55068248
网址: www.shyuanye.com
邮箱: 3008007435@qq.com

Table 1. Substrate specificity

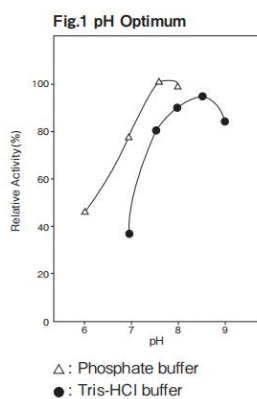
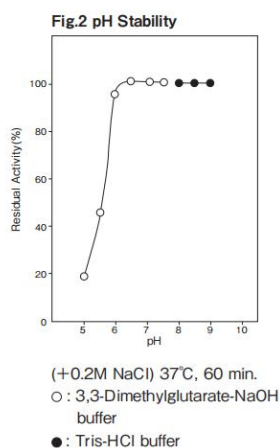
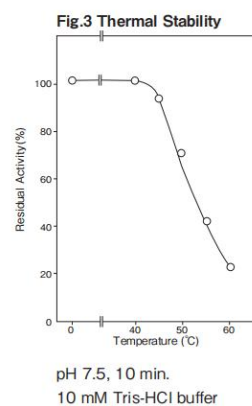
Substrate	Relative activity(%)
Methanol	100
Ethanol	79.3
n-Propanol	46.5
iso-Propanol	25.8
n-Butanol	39.6
Form aldehyde	48.2
Acetoaldehyde	0

Table 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	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

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





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 μ mole of H_2O_2 and formaldehyde from methanol per minute at 37°C under the conditions specified in the assay procedure.

Reagents

1. 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, POD

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 : A_s

blank : A_b

$$\Delta A = (A_s - A_b) \leq 0.20 \text{ Abs}$$

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.



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References:

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源叶