Sarcosine oxidase (SOX)

rp216177

Sarcosine oxidase

(EC 1.5.3.1, from Microorganism)

Sarcosine + O_2 + H_2O ⟶ Glycine+ HCHO + H_2O_2

Preparation and specification

Appearance	Yellowish amorphous powder, lyophilized
Protein purity	⩾90% (from SDS-PAGE)
Activity	⩾15 U/mg solid
Catalase	⩽0.03%
Creatinine amidohydrolase	⩽0.01%
Creatine amidohydrolase	⩽0.01%
ATPase	⩽ 0.005%

Properties

EC number	1.5.3.1	
Molecular weight	45 kDa (SDS-PAGE)	
Isoelectric point	5.3	
Michaelis constants	5.6×10^{-3} M (Sarcosine)	
Inhibitors	SDS, Zn^{2+}, Cd^{2+}	
Optimum pH	8.0	Fig. 1
Optimum temperature	50℃	Fig. 2
pH stability	pH 7.0 - 9.5 (25℃, 16 h)	Fig. 3
Thermal stability	Below 50℃ (pH 7.5, 30 min)	Fig. 4
Storage stability	At least one year at -25 ~ -15 ℃	Fig. 5

Applications

This enzyme is useful for enzymatic determination of creatinine when coupled with creatinine amidohydrolase and creatine amidohydrolase.

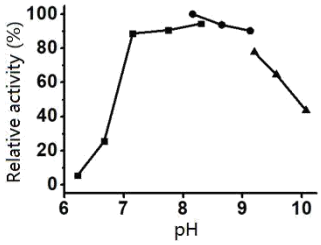


Fig. 1 Optimum pH

Buffer solution: pH 6.2-8.5, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH.

Enzyme concentration: 1 mg/mL

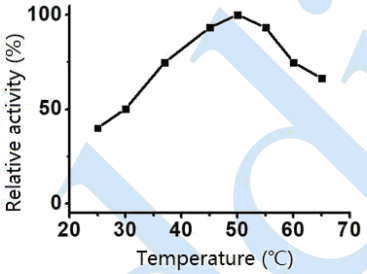


Fig. 2 Optimum temperature

Reaction in 10 mM K-phosphate buffer pH 7.5.

Enzyme concentration: 1 mg/mL

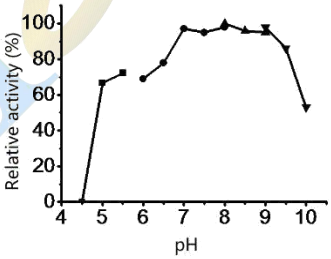


Fig. 3 pH Stability

25 ℃, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, acetate buffer; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH. Enzyme concentration: 1 mg/mL

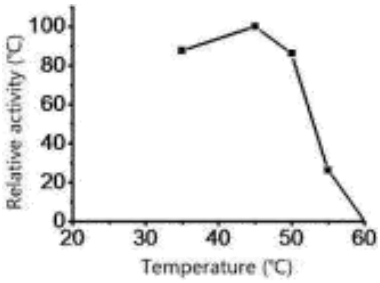


Fig. 4 Thermal stability

30 min-treatment with 100 mM K-phosphate

buffer, pH 7.5.

Enzyme concentration: 1 mg/mL

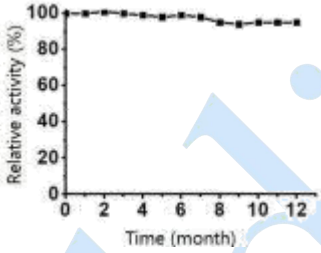
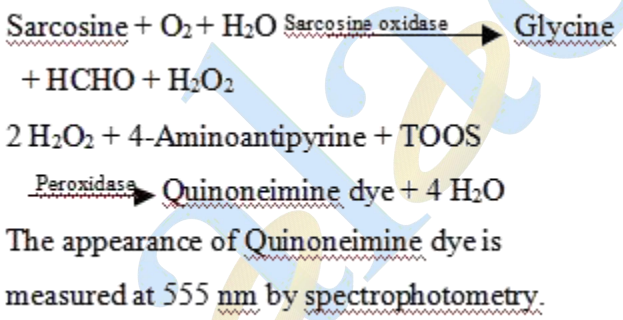


Fig.5 Storage stability (-25 ~ -15 ℃)

Assay principle



Unit definition

One unit (U) is defined as the amount of enzyme which produces 1 μmol of H_2O_2 per min under the conditions described below.

Reagents preparation

Reagent I: 0.2 M Tris-HCl buffer, pH 8.0. Reagent II: 1.0 M Sarcosine solution. Reagent III: 1

kU/mL POD solution. Reagent IV: 50 mM 4-AA solution. Reagent V: 50 mM TOOS solution.

Enzyme diluent: 10 mM KH_2PO_4 - K_2HPO_4 buffer, pH 7.5.

Enzyme solution: Dilute the enzyme to 0.07−0.17 U/ml with enzyme diluent.

Reaction mixture (50 tests):

Reagent I	5 mL
Reagent II	10 mL
Reagent III	0.25 mL
Reagent IV	1.5 mL
Reagent V	1.5 mL
Pure H_2O	31.75 mL

Procedure

1.Pipette 1 ml reaction mixture to a cuvette.
2.Preincubate the reaction mixture at 37 ℃ for 5 min.
3.Add 20 ul the enzyme solution and mix to start the reaction, record ΔAs at 555 nm in 1 minute in a spectrophotometer.
Measure the blank rate ΔAb by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.
ΔA = ΔAs - ΔAb

Calculation

$$\text{Volume activity (U/mL)} = \frac{\Delta A \times 1.02 \times df}{39.2 \times 0.020 \times t \times 1/2 \times 1.0} = \Delta A \times 2.602 \times df$$

$$\text{Weight activity (U/mg)} = \text{Volume activity} \times 1/C$$

1.02: Total volume (mL)
0.020: Enzyme volume (mL)
t: Reaction time (1min)
1.0: Light path length (cm)
1/2: 1 μmol of H_2O_2 produces 0.5 μmol of Quinoneimine dye
df: Dilution factor
C: Enzyme concentration (mg/mL)

39.2: Millimolar extinction coefficient of quinoneimine dye under 555 nm ($cm^2/\mu mol$)

References

1. N.Mori, M.Sato, Y.Tani and Y.Yamada; Agric.Biol.Chem., 44, 1391 (1980).
2. M.Suzuki and M.Yoshida; Proceedings of the Symposium on Chemical Physiolosy and Pathology (Kyoto), Vol16, p.220 (1976).
3. M.Suzuki; J. Biochem., 89, 599 (1981).