Peroxidase“Amano”3

(Donor : hydrogen-peroxide oxidoreductase, EC 1.11.1.7)

Peroxidase“Amano”3 is a peroxidase preparation extracted from horseradish.

Catalysis

Donor + H_2O_2 ⟶ Oxidized donor + $2H_2O$

Specification and Preparation

Activity:	Peroxidase activity	≧ 180 u/mg (Amano method)
Appearance:	Light brown to reddish brown powder, lyophilized	
Additive:	Not added	

Characteristics

1. Molecular weight: 41,000 (SDS-PAGE)
2. Isoelectric point: 6.2, 7.2, 8.8
3. Optimum pH: 6.0
4. pH stability: 6.0-10.0 (30°C, 30 min)
5. Optimum temperature: 40°C
6. Thermal stability: up to 60°C (pH 6.0, 1 hr)
7. Inhibitors: NaN_3, KCN
8. Application: Used for the enzymatic determination of hydrogen peroxide in clinical diagnosis.

Expiration (Storage)

24 months from the date of analysis when stored at 5°C or below in a dry place under sealed conditions.

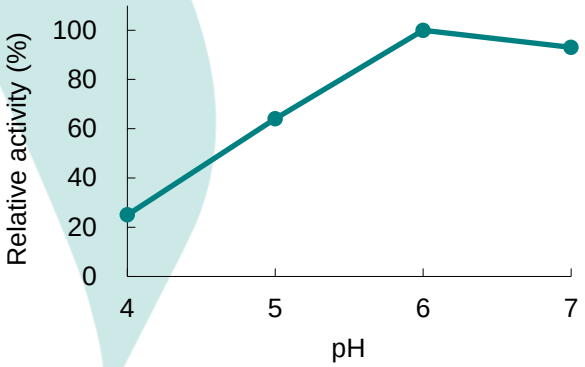
Safe Handling

1. Do not inhale.
2. In case of direct contact with skin or eyes, immediately wash or rinse with plenty of water.
3. Please refer to SDS for more details.

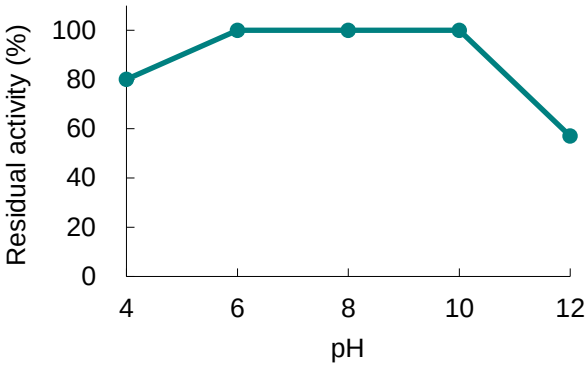
General properties

The following results demonstrate the activities of enzyme solution prepared in various buffers. Enzyme activity may vary under different experimental conditions.

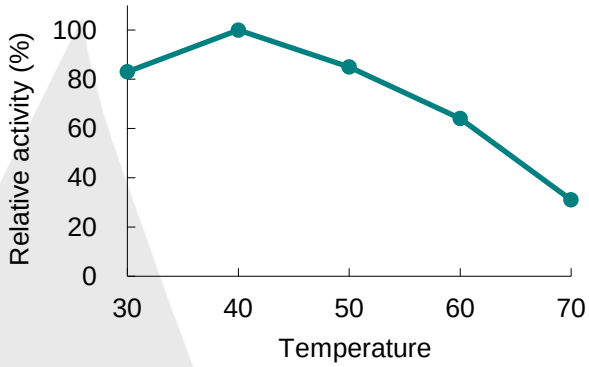
pH and Activity



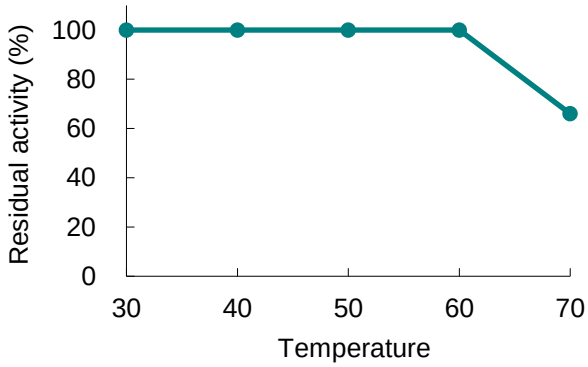
pH stability



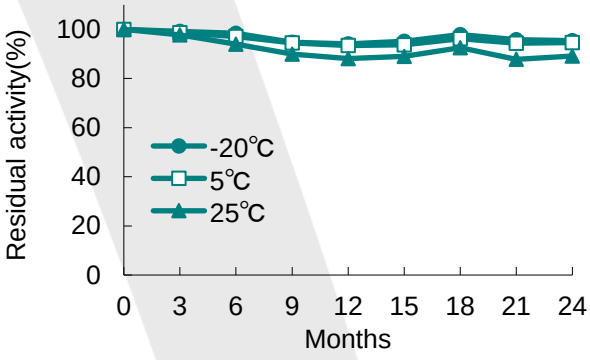
Temperature and Activity



Thermostability



Stability (powder form)



Assay method of Peroxidase activity

Principle

$2H_2O_2$ + 4-aminoantipyrine + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + $4H_2O$

The appearance of quinoneimine dye formed by coupling with 4-aminoantipyrine and phenol is measured at 500nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which decomposes one μ mole of hydrogen peroxide per minute under the conditions described below.

Reagents

A. 4-Aminoantipyrine solution (4 mg/ml deionized water)
B. Triton X-100 solution (50 mg/ml deionized water)
C. Phenol solution (50 mg/ml deionized water)
D. 0.1M Phosphate buffer (KH_2PO_4-NaOH, pH 7.0)
E. Phenol-buffer solution
Weigh 1.36g of KH_2PO_4 and dissolve in 80 ml of deionized water. Add 3 ml of Phenol solution (C) and 3 ml of Triton X-100 solution (B), then adjust the pH to 7.0 with 4N NaOH. Fill up to 100 ml with deionized water. This reagent should be used after incubation at 25℃ for 24 hours. (Can be used for 14 days at 25℃)
F. Substrate solution
Dilute 0.175 ml of hydrogen peroxide (35.5 % concentration) with deionized water and fill up to 200 ml.
G. Enzyme solution
Weigh out Peroxidase“Amano”3 and dissolve in chilled 0.1M Phosphate Buffer (D).
Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.030±0.005.

Procedure

Pipette 2.0 ml of Phenol-buffer solution (E), 1 mL of Substrate solution (F), and 0.1 ml of 4-Aminoantipyrine solution (A) respectively into quartz cell (d=10 mm). Keep at 37±0.5℃ for 10 min. Then, pipette 0.1 ml of Enzyme solution (G) into the quartz cell and mix well immediately. Keep the reaction mixture at 37±0.5℃. Exactly at 2 minutes and 5 minutes after the addition of Enzyme solution (G), measure the absorbances of the reaction mixture at 500 nm (A2 and A5).

As a blank, pipette 0.1M Phosphate buffer (D) into another quartz cell instead of the Enzyme solution (G) and follow the same procedure described above (Ab2 and Ab5).

Calculation

$$\text{Peroxidase activity (u/mg)} = \frac{(A5-A2)-(Ab5-Ab2)}{3} \times \frac{3.2}{12.88} \times 2 \times \frac{n}{0.1} \times 0.693$$

3: Reaction time
12.88: Absorption coefficient of quinoneimine dye
2: Conversion factor (1 mole of quinoneimine dye equivalent to 2 mole of hydrogen peroxide)
3.2: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution
0.693: Coefficient (to original Amano method)

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