Bilirubin Oxidase “Amano” 3

(Bilirubin : oxygen oxidoreductase, EC 1.3.3.5)

Bilirubin Oxidase “Amano” 3 is a bilirubin oxidase preparation manufactured by a unique fermentation process using a selected strain of filamentous fungi.

Catalysis

Bilirubin + 1/2 O_2 ⟶ Bilverdin + H_2O

Specification and Preparation

Activity:	Bilirubin oxidase activity	≧ 1.2 u/mg (Amano method)
Appearance:	Light blue to light bluish green powder, lyophilized	
Additives:	Saccharose, $(NH_4)_2SO_4$	

Characteristics

1. Molecular weight: 52,000 (Gel filtration)
2. Isoelectric point: 4.1
3. Km: 1.9×10^{-4} M
4. Optimum pH: 6.0-7.0
5. pH stability: 7.0-11.0 (37°C, 1hr)
6. Optimum temperature: 40-60°C
7. Thermal stability: up to 55°C (pH 7.0, 30 min)
8. Inhibitors: $FeCl_2$, NaN_3, KCN
9. Stabilizers: EDTA, Aspartic acid
10. Application: Used for the enzymatic determination of bilirubin and for eliminating the interference of bilirubin in clinical diagnosis.

Expiration (Storage)

24 months from the date of analysis when stored at -20°C or below in a dry place under sealed conditions.

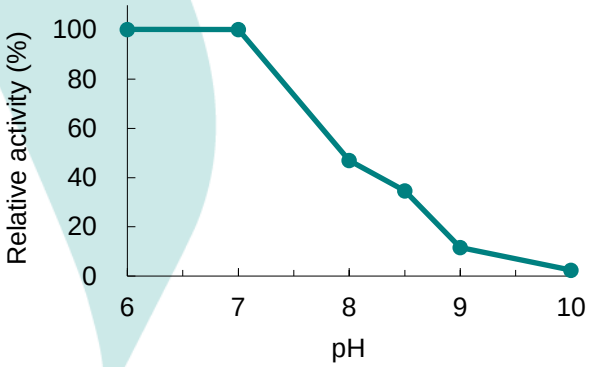
Safe Handling

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.

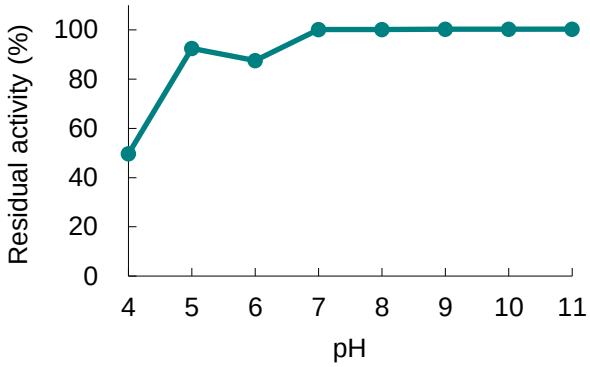
General properties

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

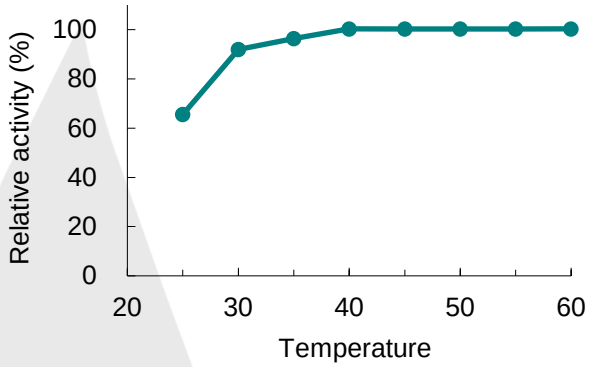
pH and Activity



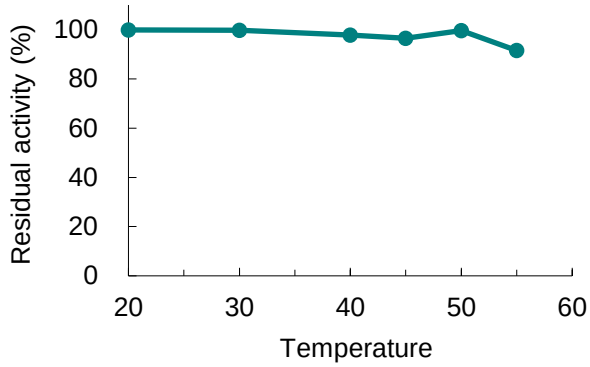
pH stability



Temperature and Activity



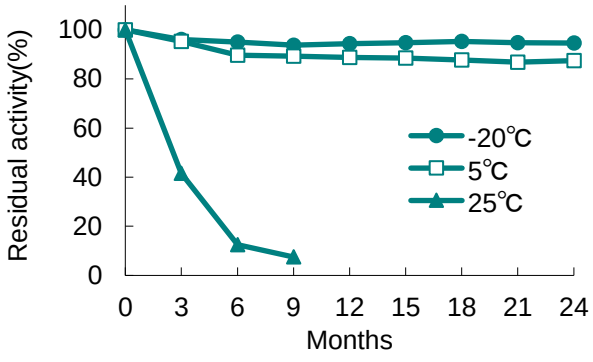
Thermostability



Effect of Various Chemicals

Chemical	Concentration (mM)	Relative Activity (%)
None	—	100
$MnCl_2$	1.0	100
$MgCl_2$	1.0	100
$CuCl_2$	0.1	100
$FeCl_2$	1.0	0
$NiCl_2$	1.0	100
$CaCl_2$	1.0	100
KCN	0.1	0
NaN_3	0.1	58
Thiourea	0.1	85

Stability (powder form)



Assay method of Bilirubin oxidase activity

Principle

$$\text{Bilirubin} + 1/2\ O_2 \xrightarrow{\text{Bilirubin oxidase}} \text{Biliverdin} + H_2O$$

The consumption of bilirubin is measured at 460 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which oxidizes one μ mole of albumin bound bilirubin per minute under the conditions described below.

Reagents

A. 0.05M Phosphate buffer (Containing EDTA, pH7.0)
Weigh 6.80 g of KH_2PO_4 and 18.6 mg of EDTA·2Na, dissolve them in approx. 800 ml of deionized water. Adjust the pH to 7.0 with 4N NaOH and fill up to 1000 ml with deionized water.

B. Sodium cholate solution
Weigh 0.50 g of sodium cholate (Nakarai Tesque Inc.) and dissolve in 0.05M phosphate buffer (A). Fill up to 50 ml with 0.05 M Phosphate buffer (A). (Can be used for 1 month if kept refrigerated)

C. Substrate solution
Weigh approx. 30 mg of Albumin bound bilirubin (Amano Enzyme Inc.) and dissolve in 1.2ml of 0.05M Phosphate buffer (A). Store in a brown bottle. (Can be used for 6hrs after preparation if kept refrigerated)
Substrate solution should be prepared so that the absorbance value at 460 nm (see the following method) is in the range of 0.730-0.740.

<A460 check method>
Pipette 3 ml of Sodium cholate solution (B) and 0.2 ml of Substrate solution (C) into a quartz cell (d = 10 mm), then measure the absorbance at 460 nm (A460). If the value exceeds the above range, Substrate solution should be remade using the weight value calculated by the following formula.

Weight value of Albumin bound bilirubin (mg) = 0.735 × 30mg ÷ A460

D. Enzyme solution
Weigh out Bilirubin Oxidase “Amano” 3 and dissolve in chilled 0.05M Phosphate buffer (A). Enzyme solution should be prepared so that the value of ΔOD/minute is in the range of 0.020±0.002.

Procedure

Pipette 3.0 ml of Sodium cholate solution (B) and 0.20 ml of Substrate solution (C) respectively into a quartz cell (d = 10 mm) and keep at 37±0.5℃ for 10 minutes. Pipette 0.10 ml of Enzyme solution (D) into the quartz cell and mix well immediately. Keep the reaction mixture at 37±0.5℃. Exactly at 1 minute and 3 minutes after the addition of Enzyme solution (D), measure the absorbances of the reaction mixture at 460 nm (A1 and A3). As a blank, pipette 0.05M Phosphate buffer (A) into another quartz cell (d = 10 mm) instead of Enzyme solution (D) and follow the same procedure described above (Ab1 and Ab3).

Calculation

$$\text{Bilirubin oxidase activity (u/mg)} = \frac{(A1-A3)-(Ab1-Ab3)}{2} \times \frac{1}{59.7} \times 3.3 \times \frac{n}{0.1}$$

2: Reaction time
59.7: Millimolar absorption coefficient of albumin bound bilirubin at 460 nm
3.3: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

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