**Xanthine Oxidase (XTOD)**

from microorganism

Xanthine : oxygen oxidoreductase, EC 1.1.3.22

\[
\text{Xanthine} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Uric acid} + \text{H}_2\text{O}_2
\]

### SPECIFICATION

- **Appearance**: White to Pail yellow lyophilizate
- **Activity**: $\geq 5.0$ U/mg
- **Contaminants**:
  - Purine-nucleoside Phosphorylase: $\leq 5 \times 10^{-3}\%$
  - Catalase: $\leq 5.0\%$
- **Stabilizer**: Glycine, Boric acid
- **Storage**: at $-20^\circ$C

### PROPERTIES

- **Molecular weight**: ca. 128 kDa (gel filtration)
- **Structure**: 2 subunits of 69 kDa (SDS-PAGE)
- **Michaelis constants**:
  - 1.1 $\times 10^{-4}$ M (xanthine)
  - 1.5 $\times 10^{-4}$ M (hypoxanthine)
- **pH Optimum**: 6.5–7.5 (Fig. 1)
- **pH Stability**: 6.5–7.0 (Fig. 2)
- **Optimum temperature**: 35–45°C (Fig. 3)
- **Thermal stability**: below 50°C (Fig. 4)
- **Inhibitors**: Cu$^{2+}$, Ag$^{2+}$, $p$-chloromercuribenzoate, L-Ascorbate
- **Specificity**: xanthine (100), hypoxanthine (95), guanine (40), adenine (0)
ASSAY PROCEDURE

Principle

\[
\text{Xanthine} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{xanthine oxidase}} \text{Uric acid} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\text{H}_2\text{O}
\]

The appearance of quinoneimine dye is measured spectrophotometrically at 5000 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 \(\mu\)mol of hydrogen peroxide per min at 37°C and pH 7.3 under the conditions described below.

Reagents

A. Tris–HCl buffer, 0.1 M; pH 7.3: dissolve 12.1 g of Tris(hydroxymethyl)aminomethane in 900 ml of distilled water, adjust to pH 7.3 with 6 N HCl and dilute with distilled water to 1000 ml.
B. Xanthine solution, 10 mM: 15.2 mg of xanthine, dissolve in 80 ml of 10 mM Tris–HCl buffer, pH 7.3 dilute with distilled water to 100 ml.
C. Phenol solution, 6.0%: 6.0 g phenol/100 ml of distilled water.
D. 4-Aminoantipyrine (4-AA) solution, 0.2%: 200 mg of 4-AA/100 ml of distilled water.
E. Peroxidase (POD) solution, 80 U/ml: 4 mg of POD (200 guaiacol U/mg)/10 ml of distilled water.
F. Enzyme dilution buffer: 10 mM Tris–HCl buffer, pH 7.3 dilute with distilled water to 100 ml.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.05–0.1 U/ml in or with ice-cold enzyme dilution buffer (Reagent E) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).
   - 2.55 ml Tris-HCl buffer (Reagent A)
   - 0.20 ml Xantine solution (Reagent B)
   - 0.05 ml 4-AA solution (Reagent C)
   - 0.10 ml Phenol solution (Reagent D)
   - 0.10 ml POD solution (Reagent E)
2. Equilibrate at 37°C for about 10 min.
3. Add 0.1 ml of sample and mix.
4. Record the increase of absorbance at 500 nm in a spectrophotometer thermostated at 37°C, and calculate the \(\Delta A\) per min using the linear portion of the curve (\(\Delta A_0\)).

The blank solution is prepared by adding enzyme dilution buffer (Reagent H) instead of sample (\(\Delta A_0\)).
Calculation

Activity can be calculated by using the following formula:

\[ \text{Volume activity (U/ml)} = \frac{(A_t - A_0) \times 3.1 \times df}{6.3 \times 1/2 \times 0.1} = \Delta A \times 9.84 \times df \]

\[ \text{Weight activity (U/mg)} = (U/ml) \times 1/C \]

6.3 : Millimolar extinction coefficient of quinoneimine dye under the assay conditions (cm²/μmol)
1/2 : Factor based on the fact that 1 mol of hydrogen peroxide produces 1/2 mol of quinoneimine dye
df : Dilution factor
C : Content of xanthine oxidase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of inorganic phosphorus in clinical analysis.

\[ \text{Pi + Inosine} \xrightarrow{\text{purine-nucleoside phosphorylase}} \text{Ribose 1-phosphate + hypoxanthine} \]

\[ \text{hypoxanthine + 2H}_2\text{O + 2O}_2 \xrightarrow{\text{xanthine oxidase}} \text{Uric acid + 2H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine + Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye + 4H}_2\text{O} \]

REFERENCES

FIG. 1  pH Optimum

Relative activity (%) vs pH

Fig. 2  pH Stability

Residual activity (%) vs pH

Fig. 3  Optimum temperature

Relative activity (%) vs °C

(Kept under dry conditions)

Buffer: 0.05 M Tris–HCl buffer, pH 7.3

Fig. 4  Thermal stability

Residual activity (%) vs °C

(Kept under dry conditions)

Treatment: 100 mM Tris–HCl buffer, pH 7.3, 60 min

Fig. 5  Stability (powder form) at 30°C

Residual activity (%) vs Week

(Kept under dry conditions)