(Diagnostic Reagent Grade)

T−129

URICASE [UODN Ⅱ]

from *Arthrobacter globiformis*
(Urate: oxygen oxidoreductase, EC 1.7.3.3)
(Urate oxidase)

$$\text{Uric Acid} + O_2 + 2\,H_2O \longrightarrow \text{Allantoin} + H_2O_2 + CO_2$$

Preparation and Specification

Appearance : White to off white amorphous powder, lyophilized
Specific activity : More than 10 U/mg solid

Properties

Molecular weight	: 117 kDa (TSK gel G3000SWXL)	
Isoelectric point	: pH 4.61	
Michaelis constant	: Uric acid 1.3 × 10−4 M	
Optimum pH	: 8.5–9.5	Figure 1
pH stability	: 8.5–9.5 (37℃ , 60 min)	Figure 2
Thermal stability	: Stable at 50℃ and below (pH 9.0, 10 min)	Figure 3
Storage stability	: At least one year at −20℃	Figure 4

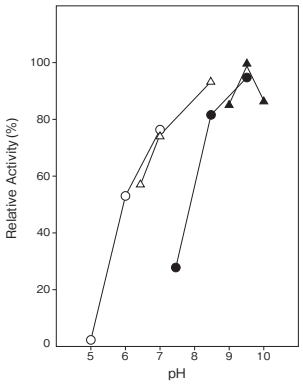
Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **uric acid**.

$$\text{Uric Acid} + O_2 + 2\,H_2O \xrightarrow{\text{UODN Ⅱ}} \text{Allantoin} + H_2O_2 + CO_2$$

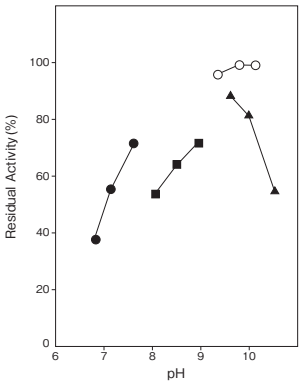
$$2\,H_2O_2 + 4\text{-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4H_2O$$

Fig.1 pH Optimum



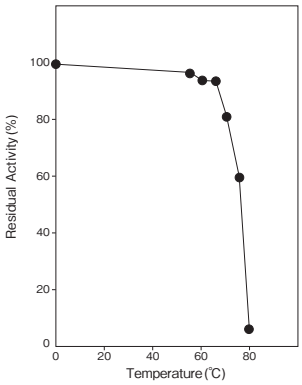
○ : 3, 3-Dimethylglutarate -NaOH buffer
△ : Phosphate buffer
● : Tris-HCl buffer
▲ : Borate buffer

Fig.2 pH Stability



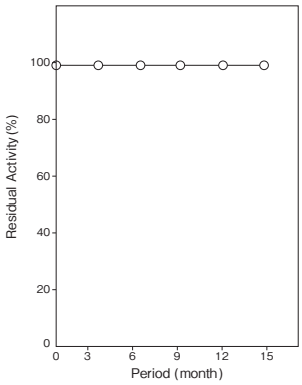
37℃, 60min
● : Phosphate buffer
■ : Tris-HCl buffer
○ : Glycine-NaOH buffer
▲ : Borate buffer

Fig.3 Thermal Stability



pH9.0 10 min.
20mM Borate buffer

Fig.4 Storage (lyophilized powder)



−20℃

Assay

■ Principle

The assay is based on the decrease in absorbance at 293 nm of uric acid which is oxidized in the following reaction:

$$\text{Uric acid} + O_2 + 2\,H_2O \xrightarrow{\text{UODN II}} \text{Allantoin} + H_2O_2 + CO_2$$

■ Unit definition

One unit is defined as the amount of enzyme which oxidizes 1 μmole of uric acid to allantoin per minute at 25℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture (0.115 mM Uric acid)
   Mix 1 ml of 3.57 mM uric acid and 30 ml of enzyme dilution buffer.
2. Enzyme dilution buffer
   20 mM sodium tetraborate–HCl buffer pH 9.0
3. Reagents
   Sodium tetraboric acid ($Na_2B_4O_7 \cdot 10H_2O$):
   FUJIFILM Wako Pure Chemical Corporation
   Special grade #194–01415
   Uric acid: Tokyo Kasei Kogyo Co., Ltd. #U0018

■ 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 3.10 ml of reaction mixture into a small test tube and preincubate at 25℃.
2. After 3 min, add 20 μl of enzyme solution and mix to start the reaction at 25℃.
   ※ In the case of a test blank, add 20 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of decrease per minute in absorbance at 293 nm. The rate must be measured within the linear portion of the absorbance curve.

   Absorbance sample : As/min
   blank : Ab/min
   △ A/min = (Ab/min − As/min) ≦ 0.060 Abs/min

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle\,\text{A/min}}{12.6} \times \frac{3.12}{0.02} \times \frac{1}{X}$$

12.6 : millimolar extinction coefficient of uric acid at 293 nm (cm^2/ μmole)
3.12 : final volume (ml)
0.02 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at − 20℃ in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 4) .

References

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UODN Ⅱ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液（0.115mM 尿酸）
   3.57mM 尿酸溶液 1ml と酵素溶解希釈用液 30ml を混合する。
2. 酵素溶解希釈用液
   20mM 四ホウ酸ナトリウム－HCl 緩衝液 pH9.0
3. 試薬
   四ホウ酸ナトリウム（$Na_2B_4O_7 \cdot 10H_2O$）：
   富士フイルム和光純薬製　特級　#194–01415
   尿酸（Uric acid）：東京化成製　#U0018

Ⅱ．酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液で溶解して全容 20ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 3.10ml を正確に分注して 25℃で予備加温する。
2. 3 分経過後、酵素試料液 20 μl を加えて混和し、25℃で反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 20 μl を加える。
3. 反応開始後、293nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求める。
   求められた吸光度を試料液は As/min、盲検液は Ab/min とする。
   Δ A/min =（As/min－Ab/min）≦ 0.060 Abs/min

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min}}{12.6} \times \frac{3.12}{0.02} \times \frac{1}{X}$$

12.6 : 尿酸の 293nm におけるミリモル分子吸光係数（$cm^2/\ \mu mole$）
3.12 : 反応総液量（ml）
0.02 : 反応に供した酵素試料液量（ml）
X　: 酵素試料液中の検品濃度（mg/ml）