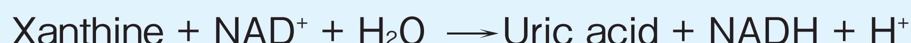
XANTHINE DEHYDROGENASE [XDH Ⅱ]

from *Microorganism*

(Xanthine: NAD^+ oxidoreductase, EC 1.17.1.4)



Preparation and Specification

Appearance : Brownish solution
Specific activity : More than 100 U/ml

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 240 kDa (TSK G-3000SW gel filtration)	
Isoelectric point	: pH 4.5 ± 0.2	
Michaelis constants	: Xanthine 2.4×10^{-4}M	
	NAD^+ 7.5×10^{-5}M	
	Thio-NAD^+ 8.0×10^{-5}M	
Optimum pH	: 8.5	Figure 1
pH stability	: 6.5–9.5 (45℃, 15 min)	Figure 2
Optimum temperature	: 55℃ (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 60℃ and below (pH 7.5, 15 min)	Figure 4
Effect of various chemicals	: See Table 2 and Table 3	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **inorganic phosphate** when coupled with purinenucleoside phosphorylase (T-69).

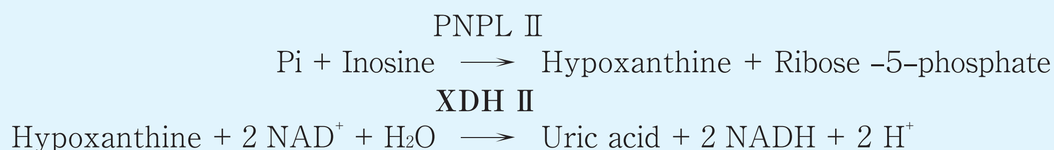


Table 1. Substrate specificity

Substrate (1mM)	Relative activity (%)
Hypoxathine	100
Xathine	75.0
Uric acid	0
Adenosine	0
Inosine	0
Xantosine	0
Guanosine	0
Adenine	0
Guanine	0
8-Azaxanthine	0
8-Azaguanine	0

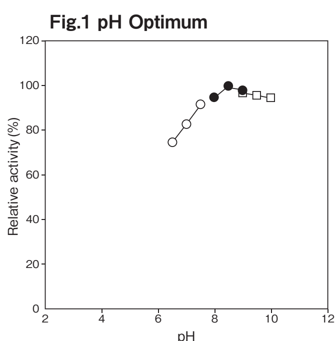
Table 2. Effect of metal ions on XDH Ⅱ activity

Metal ion	Relative activity (%)
None	100
NaCl (10mM)	100
KCl (10mM)	100
NH_4Cl (10mM)	100
CsCl (10mM)	100
LiCl (10mM)	100
$CaCl_2$ (1mM)	100
$BaCl_2$ (1mM)	92.0
$MgCl_2$ (1mM)	100
$MnCl_2$ (1mM)	88.0
$NiCl_2$ (1mM)	58.0
$CuCl_2$ (1mM)	50.0
$CoCl_2$ (1mM)	92.0

Table 3. Effect of detergents on XDH Ⅱ activity

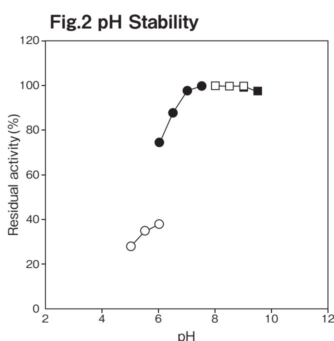
Detergent (0.5%)	Relative activity (%)
None	100
Pluronic L-71	100
Adekatol NP-690	100
Adekatol PC-8	100
Nikkol NP-18TX	100
Tween 80	100
Triton X-100	100

Fig.1 pH Optimum



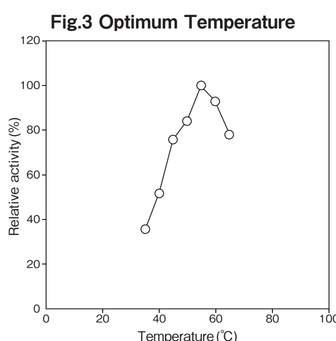
○: Phosphate buffer
●: Tris-HCl buffer
□: Glycine-NaOH buffer

Fig.2 pH Stability



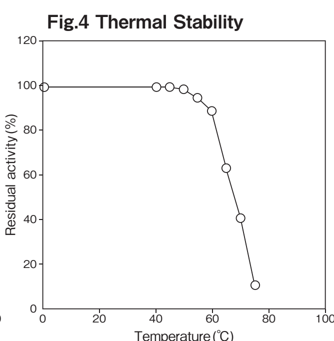
○: Acetate buffer
●: Phosphate buffer
□: Tris-HCl buffer
■: Glycine-NaOH buffer

Fig.3 Optimum Temperature



pH 7.5
100 mM Tris-HCl buffer

Fig.4 Thermal Stability

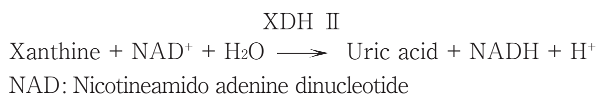


pH 7.5, 15min.
100 mM Tris-HCl buffer

Assay

■ Principle

The assay is based on the increase in absorbance at 340 nm as the formation of NADH proceeds in the following reaction:



$$\text{Xanthine} + NAD^+ + H_2O \xrightarrow{\text{XDH II}} \text{Uric acid} + NADH + H^+$$

NAD: Nicotineamido adenine dinucleotide

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μmole of xanthine to uric acid per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

1M Tris-HCl buffer pH9.0	0.30 ml
10mM NAD solution	0.60 ml
10mM Xanthine solution pH 10.5 ± 0.5 [1)]	0.60 ml

Distilled water 1.50 ml

1): 10mM Xanthine solution pH 10.5 ± 0.5

Dissolve 152 mg of xanthine with 80 ml of distilled water, adjust pH to 10.5 ± 0.5 at 25℃ with 1 N NaOH and add distilled water to make a total of 100 ml.

2. Enzyme dilution buffer

1M Tris-HCl buffer pH 8.0

3. Reagents

NAD: NACALAI TESQUE, INC. #24334-84

Xanthine: Wako Pure Chemical Industries, Ltd. #241-00013

■ Enzyme solution

Dilute accurately 0.5 ml of the sample with enzyme dilution buffer to make a 50-fold solution. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate at 37℃.
2. After 5 min, add 50 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of increase per minute in absorbance at 340 nm. The rate must be measured within the linear portion of the absorbance curve.

Absorbance sample : As/min

blank : Ab/min

$\triangle A/min = (As/min - Ab/min) \leqq 0.070\ Abs/min$

■ Calculation

$$\text{Activity (U/ml)} = \frac{\triangle A/min}{6.22} \times \frac{3.05}{0.05} \times D$$

6.22 : millimolar extinction coefficient of NADH at 340 nm (cm^2/ μmole)

3.05 : final volume (ml)

0.05 : volume of enzyme solution (ml)

D : times of dilution in enzyme solution

Storage

Storage at −20℃ in the presence of a desiccant is recommended.

XDH Ⅱ活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液

1M トリス－HCl 緩衝液 pH9.0	0.30 ml
10mM NAD 溶液	0.60 ml
10mM キサンチン溶液 pH10.5 ± 0.5 [1]	0.60 ml
精製水	1.50 ml

1)：10mM キサンチン溶液 pH10.5 ± 0.5

キサンチン 152mg を精製水 80ml で溶解した後 1N NaOH で pH10.5 ± 0.5（25℃）に調整し、精製水で全容 100ml とする。

2. 酵素溶解希釈用液

1M トリス－HCl 緩衝液 pH8.0

3. 試薬

NAD（ニコチンアミドアデニンジヌクレオチド・$3H_2O$）：ナカライテスク社製　#24334-84

キサンチン（$C_5H_4N_4O_2$）：和光純薬工業製 #241-00013

Ⅱ．酵素試料液

検品 0.5ml を酵素溶解希釈用液で 50 倍に希釈する。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 3.0ml を正確に分注して 37℃で予備加温する。
2. 5 分経過後、酵素試料液 50 μl を加えて混和し、37℃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 50 μl を加える。
3. 反応開始後、340nm 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。
   求められた吸光度変化を試料液は As/min、盲検液は Ab/min とする。
   $\Delta A/min = (As/min - Ab/min) \leqq 0.070\ Abs/min$

Ⅳ．計算

$$\text{活性 (U/ml)} = \frac{\Delta A/min}{6.22} \times \frac{3.05}{0.05} \times D$$

6.22：NADH の 340nm におけるミリモル分子吸光係数 (cm^2/ μmole)

3.05：反応総液量（ml）

0.05：反応に供した酵素試料液量（ml）

D ：酵素試料液の希釈倍率