

# PURINE NUCLEOSIDE PHOSPHORYLASE [PNPL II]

from *Bacillus* sp.

(Purine-nucleoside: orthophosphate ribosyltransferase, EC 2.4.2.1)



## Preparation and Specification

Appearance : Colorless to light brownish solution

Specific activity : More than 500 U/ml

## Properties

Molecular weight	: 280±5 kDa (gel filtration) 36±5 kDa (SDS-PAGE)	
Isoelectric point	: pH 5.3±0.2	
Michaelis constants	: Inosine $2.1 \times 10^{-4}\text{M}$ Pi $7.0 \times 10^{-5}\text{M}$	
Optimum pH	: 8.0	Figure 1
pH stability	: 6.0–10.0 (37°C, 60 min)	Figure 2
Optimum temperature	: 65°C (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 65°C and below (pH 8.5, 10 min)	Figure 4
Effect of metal ions	: See Table 1	

## Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **inorganic phosphate** when coupled with xanthine dehydrogenase (T-134).

### PNPL II



### XDH II

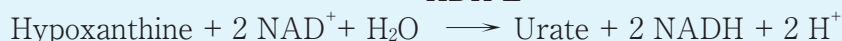
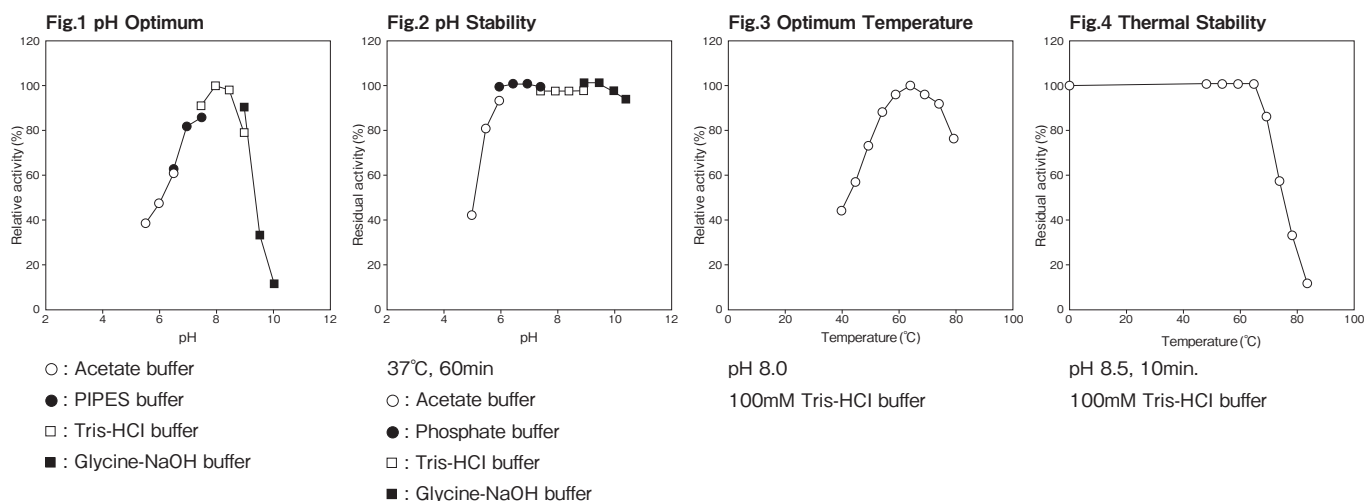


Table 1. Effect of metal ions on PNPL II activity

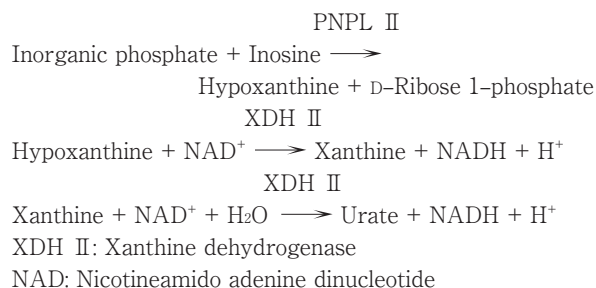
Metal ion	Relative activity (%)
None	100
KCl (10mM)	107
NaCl (10mM)	107
CsCl (10mM)	105
LiCl (10mM)	107
NH <sub>4</sub> Cl (10mM)	107
MgCl <sub>2</sub> (1mM)	107
CaCl <sub>2</sub> (1mM)	101
BaCl <sub>2</sub> (1mM)	105
MnCl <sub>2</sub> (1mM)	36.0
ZnCl <sub>2</sub> (1mM)	102
CoCl <sub>2</sub> (1mM)	24.0
CuCl <sub>2</sub> (1mM)	1.0
NiCl <sub>2</sub> (1mM)	86.0
EDTA (1mM)	112



## Assay

### Principle

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



### Unit definition

One unit is defined as the amount of enzyme which converts 1  $\mu$ mole of inosine to hypoxanthine per minute at 37°C under the conditions specified in the assay procedure.

### Reagents

1. Reaction mixture  
0.2 M Tris-HCl buffer pH 8.2 1.50 ml

- |  |         |
|--|---------|
| 10 mM NAD solution                             | 0.45 ml |
| 30 mM KH <sub>2</sub> PO <sub>4</sub> solution | 0.30 ml |
| 20 mM Inosine solution                         | 0.60 ml |
| 80 U/ml XDH II solution <sup>1)</sup>          | 0.03 ml |
| Distilled water                                | 0.12 ml |

1): 80 U/ml XDH II solution

Dissolve 800 U of XDH II with 10 ml of 20 mM Tris-HCl buffer pH 8.0 containing 5 mM EDTA.

2. Enzyme dilution buffer  
20 mM Tris-HCl buffer pH 7.5
3. Reagents  
NAD: NACALAI TESQUE, INC. #24334-84  
Inosine: FUJIFILM Wako Pure Chemical Corporation #099-00231  
EDTA: (2 Na·2 H<sub>2</sub>O) KISHIDA CHEMICAL Co., Ltd. #060-29133  
XDH II : Asahi Kasei Pharma Corporation #T-134  
EDTA: Ethylenediamine tetraacetic acid

### Enzyme solution

Dilute accurately 0.5 ml of the sample with enzyme dilution buffer to make a 50-fold solution. Dilute 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°C.
2. After 5 min, add 50  $\mu$ l of enzyme solution and mix to start the reaction at 37°C.  
※ In the case of a test blank, add 50  $\mu$ l 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

$$\Delta A/\text{min} = (As/\text{min} - Ab/\text{min}) \leq 0.045 \text{ Abs/min}$$

## ■ Calculation

$$\text{Activity (U/ml)} = \frac{\Delta A/\text{min}}{6.22 \times 2} \times \frac{3.05}{0.05} \times D$$

6.22 : millimolar extinction coefficient of NADH at 340 nm  
( $\text{cm}^2 / \mu\text{mole}$ )

2 : a multiplier derived from the fact that 1 mole of inorganic phosphate produces 2 mole of NADH.

3.05 : final volume (ml)

0.05 : volume of enzyme solution (ml)

D : times of dilution in enzyme solution

## Storage

Storage at  $-20^\circ\text{C}$  in the presence of a desiccant is recommended.

## PNPL II 活性測定法 (Japanese)

### I. 試薬液

#### 1. 反応試薬混合液

0.2M トリス-HCl 緩衝液 pH8.2	1.50 ml
10mM NAD 溶液	0.45 ml
30mM リン酸一カリ溶液	0.30 ml
20mM イノシン溶液	0.60 ml
80U/ml XDH II 溶液 <sup>1)</sup>	0.03 ml
精製水	0.12 ml

1): 80U/ml XDH II 溶液

XDH II 800 単位 (U) を 5mM EDTA を含む  
20mM トリス-HCl 緩衝液 pH8.0 10ml で溶解する。

#### 2. 酵素溶解希釈用液

20mM トリス-HCl 緩衝液 pH7.5

#### 3. 試薬

NAD (ニコチンアミドアデニンジヌクレオチド)

: ナカライテスク製 #24334-84

イノシン (Inosine):

富士フィルム和光純薬製 #099-00231

EDTA (エチレンジアミン四酢酸 $\cdot 2\text{Na}\cdot 2\text{H}_2\text{O}$ ):

キシダ化学製 #060-29133

XDH II (キサンチン脱水素酵素):

旭化成ファーマ製 #T-134

### 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°C で予備加温する。

2. 5 分経過後、酵素試料液 50  $\mu$ l を加えて混和し、37°C 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  $\mu$ l 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。

$$\Delta A/\text{min} = (As/\text{min} - Ab/\text{min}) \leq 0.045 \text{ Abs/min}$$

### IV. 計算

$$\text{活性 (U/ml)} = \frac{\Delta A/\text{min}}{6.22 \times 2} \times \frac{3.05}{0.05} \times D$$

6.22 : NADH の 340nm におけるミリモル分子吸光係数  
( $\text{cm}^2 / \mu\text{mole}$ )

2 : 無機リン 1 モルから NADH2 モルが生成することによる係数

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