(Diagnostic Reagent Grade)

T-69

PURINE NUCLEOSIDE PHOSPHORYLASE [PNPL Ⅱ]

from *Bacillus* sp.
(Purine-nucleoside: orthophosphate ribosyltransferase, EC 2.4.2.1)

Inosine + Pi ⟶ Hypoxanthine + D-Ribose 1-phosphate

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 2.1×10^{-4}M Pi 7.0×10^{-5}M	
Optimum pH	: 8.0	Figure 1
pH stability	: 6.0-10.0 (37℃, 60 min)	Figure 2
Optimum temperature	: 65℃ (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 65℃ 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).

$$\text{Pi + Inosine} \xrightarrow{\text{PNPL Ⅱ}} \text{Hypoxanthine + Ribose 1-phosphate}$$

$$\text{Hypoxanthine + 2 NAD}^+ + \text{H}_2\text{O} \xrightarrow{\text{XDH Ⅱ}} \text{Urate + 2 NADH + 2 H}^+$$

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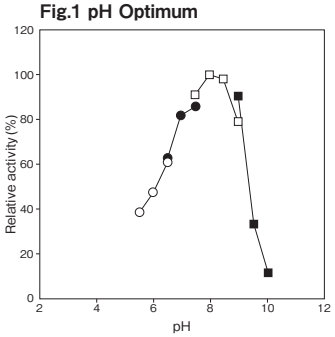
Table 1. Effect of metal ions on PNPL Ⅱ activity

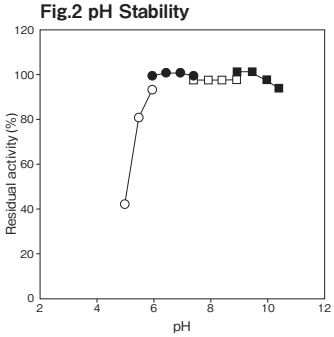
Metal ion	Relative activity (%)
None	100
KCl (10mM)	107
NaCl (10mM)	107
CsCl (10mM)	105
LiCl (10mM)	107
NH_4Cl (10mM)	107
$MgCl_2$ (1mM)	107
$CaCl_2$ (1mM)	101
$BaCl_2$ (1mM)	105
$MnCl_2$ (1mM)	36.0
$ZnCl_2$ (1mM)	102
$CoCl_2$ (1mM)	24.0
$CuCl_2$ (1mM)	1.0
$NiCl_2$ (1mM)	86.0
EDTA (1mM)	112

Fig.1 pH Optimum



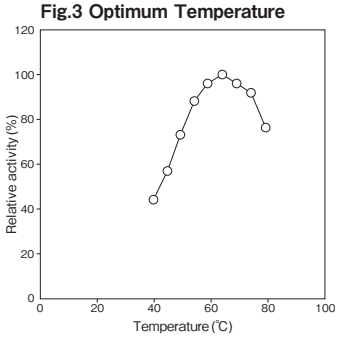
○ : Acetate buffer
● : PIPES buffer
□ : Tris-HCl buffer
■ : Glycine-NaOH buffer

Fig.2 pH Stability



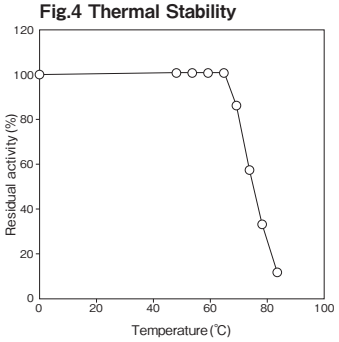
37℃, 60min
○ : Acetate buffer
● : Phosphate buffer
□ : Tris-HCl buffer
■ : Glycine-NaOH buffer

Fig.3 Optimum Temperature



pH 8.0
100mM Tris-HCl buffer

Fig.4 Thermal Stability



pH 8.5, 10min.
100mM 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 reactions:

$$\text{Inorganic phosphate} + \text{Inosine} \xrightarrow{\text{PNPL Ⅱ}} \text{Hypoxanthine} + \text{D-Ribose 1-phosphate}$$

$$\text{Hypoxanthine} + NAD^+ \xrightarrow{\text{XDH Ⅱ}} \text{Xanthine} + NADH + H^+$$

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

XDH Ⅱ: Xanthine dehydrogenase
NAD: Nicotineamido adenine dinucleotide

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μmole of inosine to hypoxanthine per minute at 37℃ 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_2PO_4 solution	0.30 ml
20 mM Inosine solution	0.60 ml
80 U/ml XDH Ⅱ solution [1)]	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_2O) KISHIDA CHEMICAL Co., Ltd. #060-29133
XDH Ⅱ : Nagase Diagnostics Co., Ltd. #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.

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■ 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

△A/min = (As/min − Ab/min) ≦ 0.045 Abs/min

■ Calculation

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

6.22 : millimolar extinction coefficient of NADH at 340 nm (cm^2 / μmole)
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℃ 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 1)	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) :
富士フイルム和光純薬製 #099-00231
EDTA (エチレンジアミン四酢酸・2Na・$2H_2O$) :
キシダ化学製 #060-29133
XDH Ⅱ (キサンチン脱水素酵素) :
ナガセダイアグノスティックス製 #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℃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。
   ΔA/min = (As/min − Ab/min) ≦ 0.045 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 (cm^2 / μmole)
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

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