

SPECIFICATION SHEET

Product Name: Purine-Nucleoside Phosphorylase
Catalog # PP01D1-E311H

Product Description: This enzyme is a diagnostic grade reagent from a microorganism host. It is useful for enzymatic determination of inorganic phosphate, 5'-nucleotidase and adenosine deaminase when coupled with xanthine oxidase and uricase.
 EC 2.4.2.1

Storage and Stability: Product can be stored at 2~8°C for transportation process up to ten days but long-term storage should be at -20°C.

Principle:

Inosine + Pi $\xrightarrow{\text{purine-nucleoside phosphorylase}}$ Hypoxanthine + α -D-Ribose 1 - phosphate
 Hypoxanthine + 2H₂O + 2O₂ $\xrightarrow{\text{xanthine oxidase}}$ Uric acid + 2H₂O₂
 The appearance of uric acid is measured at 293 nm by spectrophotometry.

Unit Definition	: One unit causes the formation of one micromole of uric acid per minute at pH 7.7 at 37°C.	
Appearance	: White amorphous powder, lyophilized	
Activity	: ≥ 30 U/mg solid	
Contaminants	: Catalase	$\leq 20\%$
	: ATPase	$\leq 1.0 \times 10^{-2} \%$
	: 5'-Nucleosidase & Adenosine deaminase	$\leq 1.0 \times 10^{-3} \%$
Molecular weight	: 30 kDa	
Isoelectric point	: 5.83	
Michaelis constant	: 7.33×10^{-5} M (Inosine), 1.05×10^{-4} M (Pi)	
Inhibitors	: Co ³⁺ , Cu ²⁺ , Hg ²⁺ , Ag ⁺ , SDS, NEM, IAA, ProClin-300	
Optimum pH	: 6.5-7.5	(Figure 1)
Optimum temperature	: 55~65°C	(Figure 2)
pH stability	: pH 6.0~10.5 (30°C, 16 hr)	(Figure 3)
Thermal stability	: below 65°C (pH 7.7, 30 min)	(Figure 4)
Effect of various chemicals	: (Table 1)	

Table 1. Effect of Various Chemicals on Purine-Nucleoside Phosphorylase

The enzyme solution dissolved in 50 mM PIPES buffer, pH 7.7 (10 U/mL) was incubated with each chemical at 30°C for 1 hr.

Chemical	Conc. (mM)	Residual activity (%)	Chemical	Conc. (mM)	Residual activity (%)
None	—	100.0	IAA	2	42.8
CaCl ₂	2	93.3	Hydroxylamine	2	97.6
MgSO ₄	2	85.4	EDTA	5	97.1
ZnSO ₄	2	100.7	NaF	20	86.2
NiCl ₂	2	92.0	NaN ₃	20	84.9
CoCl ₂	2	78.2	Proclin-300	0.045% (v/v)	19.3
MnCl ₂	2	86.1	SDS	0.05% (w/v)	1.4
FeCl ₃	2	86.1	Na-Cholate	0.1% (w/v)	109.0
CuSO ₄	2	7.6	Tween-20	0.1% (v/v)	111.2

AgNO₃ 2 0.1

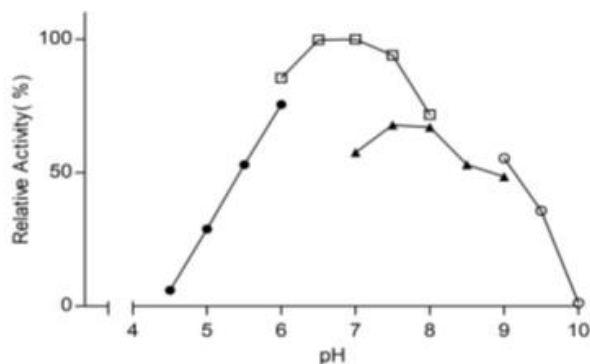
Triton X-100 0.1% (v/v) 111.6

Table 1(Continued)

Chemical	Conc. (mM)	Residual activity (%)
HgSO ₄	2	0
NEM	2	0.6
BME	2	94.3

Chemical	Conc. (mM)	Residual activity (%)
Span-20	0.1% (v/v)	97.0
Brij-35	0.1% (v/v)	112.5

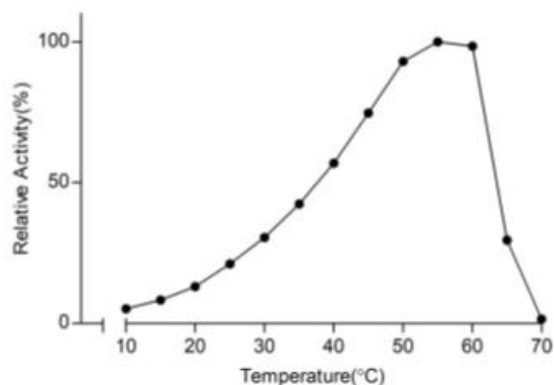
Fig.1. pH Activity



37°C in the following buffer solution:

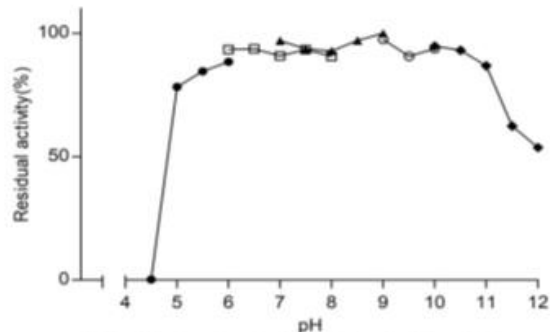
- 50mM Acetate buffer
- 50mM K-phosphate buffer
- ▲ 50mM Tris-HCl buffer
- 50mM Glycine-NaOH buffer

Fig.2. Temperature Activity



in 50mM K-phosphate buffer, pH 7.7

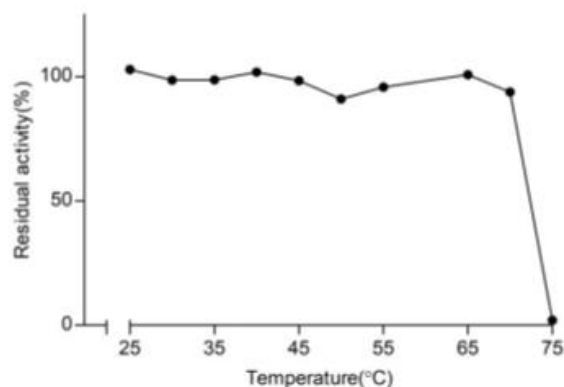
Fig.3. pH Stability



30°C, 16hr-treatment with following buffer solution:

- 50mM Acetate buffer
- 50mM K-phosphate buffer
- ▲ 50mM Tris-HCl buffer
- 50mM Borate buffer
- ◆ 50mM Glycine-NaOH buffer

Fig.4. Thermal Stability



30min- treatment with 50mM K-phosphate buffer, pH7.7

