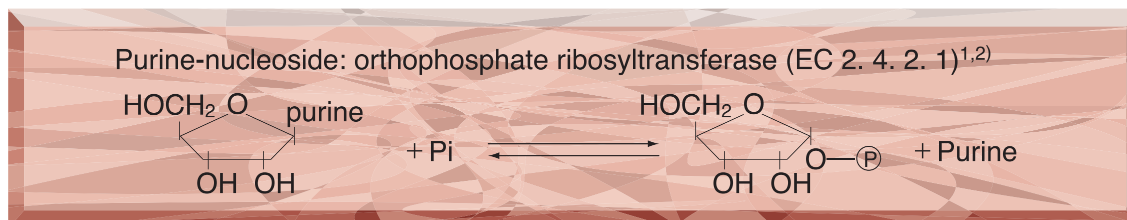
●TOYOBO ENZYMES●
(Diagnostic Reagent Grade)

# PURINE-NUCLEOSIDE PHOSPHORYLASE

*from Microorganism*




## PREPARATION and SPECIFICATION

| | |
|---|---|
| Appearance | : White amorphos powder, lyophilized |
| Activity | : Grade Ⅲ 15U/mg-solid or more |
| Contaminants | : Catalase ≤20% |
| | 5'-Nucleosidase ≤$1.0\times10^{-3}$% |
| | Adenosine deaminase ≤$1.0\times10^{-3}$% |
| | ATPase ≤$1.0\times10^{-2}$% |
| Stabilizers | : K-Gluconate, mannitol, EDTA |

## PROPERTIES

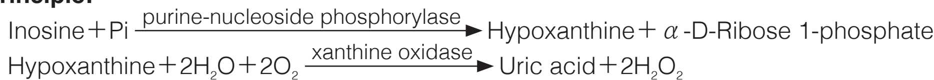
| | | |
|---|---|---|
| Stability | : Stable at −20℃ for at least one year | (Fig.1) |
| Molecular weight | : approx. 120,000 | |
| Isoelectric point | : 4.1±0.1 | |
| Michaelis constant | : $6.4\times10^{-5}$M (Inosine), $3.2\times10^{-4}$M (Pi) | |
| Inhibitors | : p-Chloromercuribenzoate, SDS, $Hg^{++}$, $Ag^{+}$ | |
| Optimum pH | : 7.5−8.0 | (Fig.3) |
| Optimum temperature | : 65℃ | (Fig.4) |
| pH Stability | : pH 6.0−9.0 (30℃, 16hr) | (Fig.5) |
| Thermal stability | : below 60℃ (pH 7.7, 30min) | (Fig.6) |
| Substrate specificity | : (Table 1) | |
| Effect of various chemicals | : (Table 2) | |

## APPLICATIONS [3~5)]

This enzyme is useful for enzymatic determination of inorganic phosphorus, 5'-nucleotidase and adenosine deaminase when coupled with xanthine oxidase (XTO-212) and uricase (UAO-201, UAO-211)

## ASSAY

**Principle:**




The appearance of uric acid is measured at 293nm by spectrophotometry.

**Unit definition:**

One unit causes the formation of one micromole of uric acid per minute under the conditions describe below.

**Method:**

Reagents

A. K-Phosphate buffer, pH 7.7 : 50mM

B. Inosine solution : 32mM [Dissolve 85.8mg of inosine (MW=268.23) in 10ml of $H_2O$ with heating] (Stable for at least two weeks if stored at 4℃)

C. Xanthine oxidase solution : ca.6.6U/ml [Dissolve xanthine oxidase (XTO-212) to ca.6.6U/ml with ice-cold buffer A] (Should be prepared fresh)

D. Enzyme diluent : buffer A

Procedure

1. Prepare the following reaction mixture in a cuvette (d=1.0cm) and equilibrate at 37℃ for about 5 minutes.

| | | |
|---|---|---|
| 2.7ml | K-Phosphate buffer, pH 7.7 | (A) |
| 0.2ml | Substrate solution | (B) |
| 0.1ml | Xanthine oxidase solution | (C) |

| Concentration in assay mixture | |
|---|---|
| K-Phosphate buffer | ca.47 mM |
| Inosine | 2.1 mM |
| Xanthine oxidase | ca. 0.2U/ml |

2. Add 0.05ml of the enzyme solution* and mix by gentle inversion.
3. Record the decrease in optical density at 293nm against water for 3 to 4 minutes in a spectrophotometer thermostated at 37℃, and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).

   At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent (D), and dilute to 0.1−1.5U/ml with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{12.5 \times 1.0 \times Vs} = \Delta OD/min \times 4.88 \times df$$

Weight activity (U/mg) = (U/ml) × 1/C

Vt : Total volume (3.05ml)

Vs : Sample volume (0.05ml)

12.5 : Millimolar extinction coefficient of uric acid under the assay condition ($cm^2$/micromole)

1.0 : Light path length (cm)

df : Dilution factor

C : Enzyme concentration in dissolution (c mg/ml)

## REFERENCES

1) R.E.Parks, Jr. and R.P.Agarwal; *The Enzymes, Vol.7,* p483 (3rd ed.)(1972)
2) P.A.Hoffe, R.May and B.C.Robertson; *Methods in Enzymology, Vol.11,* p70 (1972)
3) Y.Machida and T. Nakanishi; *Agric.Biol.Chem., 45,* 1801 (1981)
4) M.Sugiura, K.Kato, T.Adachi, Y.Ito, K.Hirano and S.Sawaki; *Chem.Pham.Bull., 29,* 1451 (1981)
5) P.Fossati; *Analytical Biochemistry., 149,* 62 (1985)

Table 1. Substrate Specificity of Purine-nucleoside phosphorylase[3)]
[Inosine: Purine-nucleoside phosphorylase - Xanthine oxidase system, pH 7.7
Guanosine, Adenosine, ATP, Thymidine: UV-system, pH 7.4]

| Substrate(0.2mM) | Relative activity(%) | Substrate(0.2mM) | Relative activity(%) |
|---|---|---|---|
| Inosine | 100 | ATP | 0 |
| Guanosine | 41 | Thymidine | 0 |
| Adenosine | 0 | | |

Table 2. Effect of Various Chemicals on Purine-nucleoside phosphorylase
[The enzyme dissolved in 50mM PIPES buffer, pH 7.0 (10U/ml) was incubated with each chemical at 30℃ for 1hr.]

| Chemical | Concn.(mM) | Residual activity(%) |
|---|---|---|
| None | — | 100 |
| Metal salt | 2.0 | |
| $MgCl_2$ | | 90.1 |
| $CaCl_2$ | | 96.7 |
| $Ba(OAc)_2$ | | 93.4 |
| $FeCl_3$ | | 73.9 |
| $MnCl_2$ | | 95.0 |
| $ZnCl_2$ | | 77.6 |
| $NiCl_2$ | | 94.1 |
| $CuSO_4$ | | 9.8 |
| $Pb(OAc)_2$ | | 9.1 |
| $AgNO_3$ | | 0.5 |
| $HgCl_2$ | | 0.1 |

| Chemical | Concn.(mM) | Residual activity(%) |
|---|---|---|
| N-ethylmaleimide | 2.0 | 91.9 |
| NaF | 2.0 | 90.9 |
| $NaN_3$ | 20 | 95.7 |
| EDTA | 5.0 | 96.8 |
| o-Phenanthroline | 2.0 | 98.3 |
| Borate | 50 | 9.0 |
| Iodoacetamide | 2.0 | 98.7 |
| Triton X-100 | 0.10% | 138.4 |
| Na-cholate | 0.10% | 124.4 |
| SDS | 0.10% | 0.1 |
| Span 20 | 0.10% | 128.9 |

EDTA, Ethylenediaminetetraacetate; SDS, Sodium dodecyl sulfate.

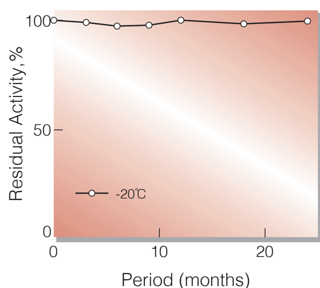



Fig.1. Stability (Powder form)
〔kept under dry conditions〕

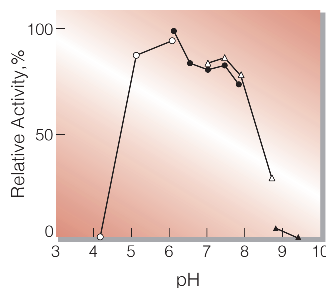



Fig.3. pH-Activity
[in 50mM buffer solution: pH 4-6, acetate; pH 6-8, K-phosphate; pH 7-9, Tris-HCl; pH 9-10, Borate]

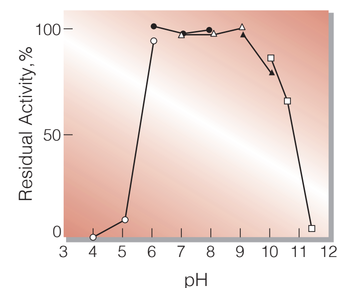



Fig.5. pH-Stability
[30℃, 16hr-treatment with 50mM buffer solution: pH 4-6, Acetate; pH 6-8, K-phosphate; pH 7-9, Tris-HCl; pH 9-10, Borate; pH10-12, Glycine-NaOH.
Enzyme concentration: 10U/ml]

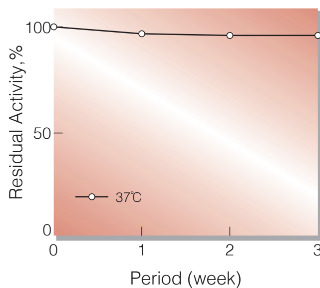



Fig.2. Stability (Powder form)
〔kept under dry conditions〕

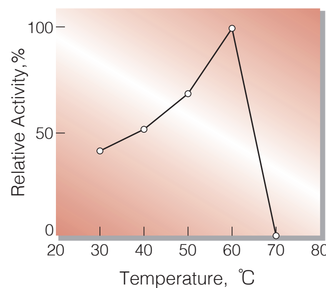



Fig.4. Temperature activity
〔in 50mM K-phosphate buffer, pH 7.7〕

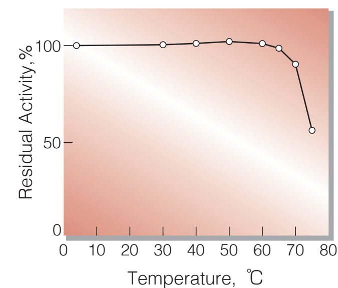



Fig.6. Thermal stability
[30min-treatment with 50mM K-phosphate buffer, pH 7.7.
Enzyme concentration: 1U/ml]

## 活性測定法（Japanese）

### 1.原理

Inosine＋Pi $\xrightarrow{\text{purine-nucleoside phosphorylase}}$ Hypoxanthine＋α-D-Ribose 1-phosphate

Hypoxanthine＋$2H_2O$＋$2O_2$ $\xrightarrow{\text{xanthine oxidase}}$ Uric acid＋$2H_2O_2$

尿酸の生成量を293nmにおける吸光度の変化で測定する。

### 2.定義

下記条件下で1分間に1マイクロモルの尿酸を生成する酵素量を1単位(U)とする。

### 3.試薬

A.　50mM K-リン酸緩衝液，pH 7.7

B.　32mM イノシン水溶液〔85.8㎎のイノシン(MW＝268.23)を10㎖の蒸留水に加温溶解する〕(4℃保存で2週間は使用可能)

C.　キサンチンオキシダーゼ溶液〔Roche製硫安懸濁結晶酵素(約20U/㎖)を氷冷緩衝液Aで約6U/㎖に希釈する〕(用時調製)

酵素溶液：酵素標品を予め氷冷した50mM K-リン酸緩衝液，pH7.7で溶解し，同緩衝液で0.1～1.5U/㎖に希釈して氷冷保存する。

### 4.手順

①下記反応混液をキュベット(d=1.0cm)に調製し,37℃で約5分間予備加温する。

| | | |
|---|---|---|
| 2.7 ㎖ | K-リン酸緩衝液 | (A) |
| 0.20㎖ | 基質溶液 | (B) |
| 0.10㎖ | キサンチンオキシダーゼ溶液 | (C) |

②酵素溶液0.05㎖を添加し,ゆるやかに混和後,水を対照に37℃に制御された分光光度計で293nmの吸光度変化を3～4分間記録し,その初期直線部分から1分間当たりの吸光度変化を求める(ΔODtest)。

③盲検は反応混液①に酵素溶液の代わりに酵素希釈液(50mM K-リン酸緩衝液,pH7.7)を0.05㎖を加え,上記同様に操作を行って,1分間当たりの吸光度変化を求める(ΔODblank)。

### 5.計算式

$$\text{U/㎖} = \frac{\Delta\text{OD/min }(\Delta\text{OD test}-\Delta\text{OD blank})\times 3.05(\text{㎖})\times\text{希釈倍率}}{12.5\times 1.0\times 0.05(\text{㎖})}$$

$$= \Delta\text{OD/min}\times 4.88\times\text{希釈倍率}$$

U/mg ＝U/㎖×1/C

12.5　：尿酸のミリモル分子吸光係数(㎠/micromole)

1.0　：光路長(cm)

C　：溶解時の酵素濃度(c mg/㎖)

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