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**Purine nucleoside phosphorylase (PNP, EC2.4.2.1)** is also known as PNPase and inosine phosphorylase. In enzymology, a purine nucleoside phosphorylase is an enzyme that catalyzes the chemical reaction, and the two substrates of this enzyme are purine nucleoside and phosphate, whereas its two products are purineand alpha-D-ribose-1-phosphate.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + NH4^+$$

$$\text{Inosine} + \text{Pi} \xrightarrow{\text{PNP}} \text{Hypoxanthine} + \text{Ribose-1-Phosphate}$$

Adenosine is first metabolized to inosine via the enzyme adenosine deaminase.Nucleoside phosphorylase is an enzyme which cleaves a nucleoside by phosphorylating the ribose to produce a nucleobase and ribose-1-phosphate. It is one enzyme of the nucleotide salvage pathways.

# Purine nucleoside phosphorylase (PNP)

A new generation of purine nucleoside phosphorylase was recently produced by CUSAg. The enzyme can be used for adenosine deaminase assay, which is a simple, rapid, sensitive and homogeneous assay that can be performed using automated systems for high-throughput assays.

| Characters | Parameters |
|---|---|
| Appearance: | White amorphous powder, lyophilized |
| Activity: | ≥ 80 U/mg -solid or more |
| Genus: | Microorganism |
| Purification method, Purity: | Chromatography, etc, > 90% (SDS-PAGE) |
| Stability: | Store at -20°C |
| Michaelis constants: | $8.16\times10^{-5}$M (Inosine, 37°C) |
| Optimum pH: | 8.0-8.5 |
| Optimum temperature: | 55°C |
| pH Stability: | pH 9.5-10.0 (37°C, 16h) |
| Thermal stability: | ≤ 65°C (pH 7.5, 15 min) |
| Inhibitors: | $Na^+$ , etc (activator) |
| Catalog Number: | CSB-DE012 |

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## 1 Thermal stability

◎**Thermal stability of PNP-solid:** PNP-solid were stored at -20°C and 37°C for 14 days, respectively. During this period, enzyme activity of PNP was determined on our biochemistry platform.Fig.1 shows that the enzymatic activity of PNP incubated at 37°C is closed to that at -20°C, representing that our CUSAg enzyme was stable from -20°C to 37°C.

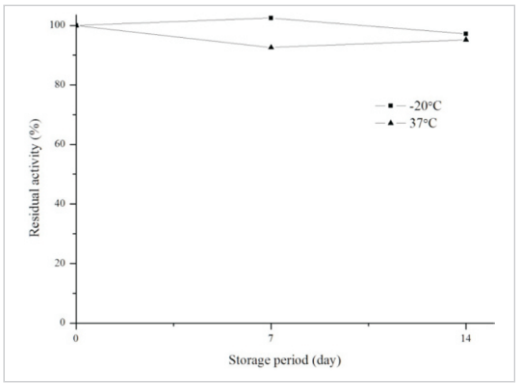


Fig.1 Thermal stability of PNP-solid

## 2 Clinical comparison

Our CUSAg adenosine deaminase reagent was also evaluated in medium-scale clinical trials with random blood samples from donations (n=82). Fig.2 shows that the correlation coefficient (r) is as high as 0.99 between in-house biochemistry assay and commercial assay. These results show good agreement between the two systems.

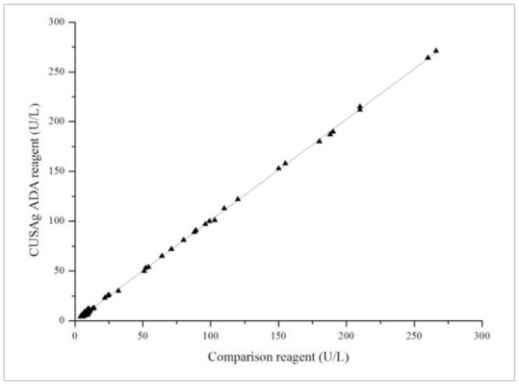


Fig.2 Clinical comparison of in-house adenosine deaminase reagent and commercial kit

## 3 Accuracy

One level of adenosine deaminase control was analyzed in replicates of three at two separated time using our two lots of reagents on the CUSAg biochemistry platform. Data from this study are summarized in the below table.1, the relative bias was <2%.

Table.1 Accuracy of adenosine deaminase reagent

| Control | Control Target value (U/L) | Determined Con. (U/L) | | | Mean Con. (U/L) | Bias (%) |
|---|---|---|---|---|---|---|
| 1 | 40.0 | 40.0 | 40.0 | 40.0 | 40.0 | +0.0 |
| 2 | 40.0 | 40.0 | 39.0 | 39.0 | 39.3 | -1.6 |

## 4 Precision

Two members of serum samples based panel were assayed, using a single lot of reagents, in replicates of ten on the CUSAg biochemistry platform (from 4 to 22 U/L as normal). As shown in table.2, the system shows excellent precision with CV≤6%.

Table.2 Precision profile of adenosine deaminase reagent

| Panel Member | n | Mean Con.(U/L) | SD | CV(%) |
|---|---|---|---|---|
| 1 | 10 | 6.1 | 0.3 | 5.2 |
| 2 | 10 | 281.8 | 1.4 | 0.5 |

## 5 Enzymatic properties

◎**Optimum pH:** Enzyme activity of PNP was determined at 37°C in different kinds of buffer (50 mM buffer: pH 4.0-5.0 Na-phosphate-citrate; pH 5.5-7.5 K-phosphate; pH 8.0-9.0 Tris-HCl; pH 9.5-11.0 CAPS-NaOH). As shown in Fig.3, the optimum pH of PNP is from 8.0 to 8.5.

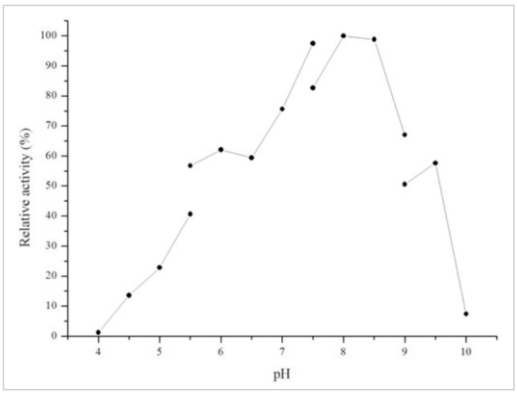


Fig.3 Optimum pH

(at 37°C in the 50mM buffer solution: pH 4.0-5.0 Na-phosphate-citrate; pH 5.5-7.5 K-phosphate; pH 8.0-9.0 Tris-HCl; pH 9.5-11.0 CAPS-NaOH)

◎ **Optimum temperature:** The enzyme activity of CUSAg PNP was determined at different temperatures in 50 mM K-phosphate pH 7.7. Fig.4 shows that the optimum temperature of PNP is 55°C.

PNP

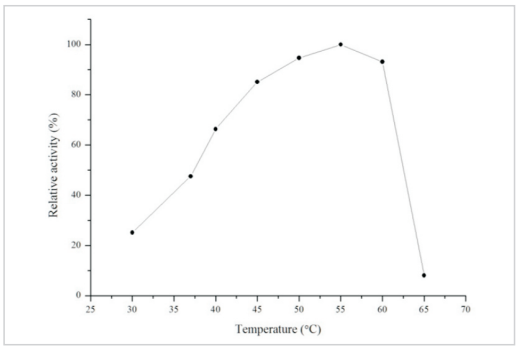


Fig.4 Optimum temperature

(50 mM K-phosphate, pH 7.7)

◎ **pH Stability:** 0.7 U/mL of CUSAg PNP was incubated at 30°C for 16 hours in different buffer (50 mM buffer: pH 4.0-5.5 Na-phosphate-citrate; pH 6.5-7.5 K-phosphate; pH 8.0-9.0 Tris-HCl; pH 9.5-10.0 CAPS-NaOH; pH 10.5-11.5 Na-phosphate-NaOH), and the residual activities were determined with biochemistry assay at 37°C. As shown in Fig.5, PNP is stable from pH 9.5 to 10.0.

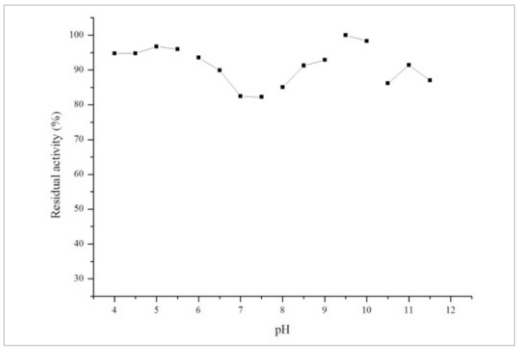


Fig.5 pH stability

(16 hours -treatment at 30°C in the 50 mM buffer solution: pH 4.0-5.5 Na-phosphate-citrate; pH 6.5-7.5 K-phosphate; pH 8.0-9.0 Tris-HCl; pH9.5-10.0 CAPS-NaOH; pH 10.5-11.5 Na-phosphate-NaOH; enzyme concentration: 0.7 U/mL)

◎ **Thermal stability:** Our CUSAg PNP was incubated from 30°C to 80°C for 15 minutes in 50 mM K-phosphate buffer (pH 7.5), containing 0.7 U/mL of PNP, and the residual activities were determined with colorimetric assay on bio-chemical analyzer. As shown in Fig.6, PNP is stable below 65°C.

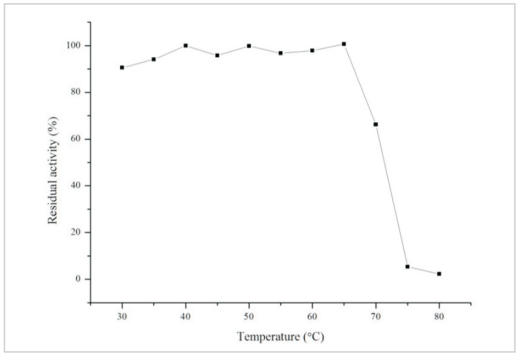


Fig.6 Thermal stability

(15 min-treatment with 50mM K-phosphate buffer, pH 7.5, enzyme concentration: 0.7 U/mL)

◎ **Effect of Various Chemicals:** CUSAg PNP was dissolved at concentration of 1.35 U/mL in 50 mM, pH 8.0, Tris-HCl buffer, which was incubated with each chemical at 37°C for 20 min. The activities were determined with biochemistry assay. As shown in table.3, PNP can be activated by $Na^+$.

Table.3 Effect of Various Chemicals on PNP

| Chemical | Concn.(mM) | Residual activity (%) |
|---|---|---|
| None | — | 100 |
| NaCl | 5.0 | 120 |
| $CuSO_4$ | 5.0 | 103 |
| $CaCl_2$ | 5.0 | 103 |
| KCl | 5.0 | 100 |
| $NH_4Cl$ | 5.0 | 105 |
| $MnCl_2$ | 5.0 | 99 |
| $FeCl_3$ | 5.0 | 112 |
| $MgSO_4$ | 5.0 | 100 |
| $NiCl_2$ | 5.0 | 112 |
| $NaN_2$ | 5.0 | 95 |

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