POLYNUCLEOTIDE PHOSPHORYLASE (PNPase)

[EC 2. 7. 7. 8]

from *Bacillus stearothermophilus*

\[ \text{RNA}_{n+1} + \text{Pi} \leftrightarrow \text{RNA}_n + \text{Nucleoside diphosphate} \]

**FOR DEPOLYMERIZATION REACTION**

**SPECIFICATION**
- State: Lyophilized
- Specific activity: more than 2,000 U/mg protein

**PROPERTIES**
- Molecular weight: 300,000 - 340,000
- Subunit molecular weight: ca. 85,000
- Optimum pH: 9.0 - 9.5
- pH stability: 9.0 - 11.0
- Isoelectric point: 4.0
- Thermal stability: No detectable decrease in activity up to 55 °C
- Michaelis constants: (38 mM Tris-HCl buffer, pH 9.5, at 60 °C)
  - Poly A: 0.27 mM**
  - KH$_2$PO$_4$: 3.0 mM
- **concentration of poly A was calculated as AMP concentration
- Effectors: cations and anions

**STORAGE**
- Stable at -20 °C for at least one year

**APPLICATION**
- The enzyme is useful for the preparation of polyribonucleotide.
ASSAY

Principle
The change in absorbance is measured at 340 nm according to the following reactions.

\[
\text{Poly A}_n + \text{Pi} \xrightarrow{\text{PNPase}} \text{Poly A}_{n-1} + \text{ADP} \quad (I)
\]
\[
\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{ATP} + \text{Pyruvate}
\]
\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ \quad (II)
\]

Unit Definition
One unit of activity is defined as the amount of PNPase that forms 1 μmol of ADP per hour at 60 °C by depolymerizing of Poly A.

Solutions
(Reaction I)
I Buffer solution ; 100 mM Tris-HCl, pH 9.5 ((1.212 g Tris + 0.074 g EDTA + 0.014 mL 2-mercaptoethanol + 0.610 g MgCl$_2$·6H$_2$O + 0.746 g KCl)/80 mL distilled water, adjusted to pH 9.5 with 1 N-HCl and filled up to 100 mL with distilled water)
II KH$_2$PO$_4$ solution ; 65 mM (0.088 g KH$_2$PO$_4$/10 mL distilled water)
III polyadenylate (Poly A) solution ; (25 mg Poly A potassium salt/1 mL distilled water; ca. 35 mM based on AMP concentration)

(Reaction II)
IV Buffer solution ; 100 mM Triethanolamine buffer, pH 7.6 ((9.300 g triethanolamine-HCl + 0.407 g MgCl$_2$·6H$_2$O + 0.373 g KCl)/400 mL distilled water, adjusted to pH 7.6 with 1 N-NaOH and filled up to 500 mL with distilled water)
V NADH solution ; 13.1 mM (0.100 g NADH disodium salt·3H$_2$O/10 mL distilled water)
VI Phosphoenolpyruvate (PEP) solution ; 56mM (0.150 g PEP MCA salt/10 mL distilled water)
VII Pyruvate kinase (PK) ; (from rabbit muscle, Roche Diagnostics K.K., No. 128 155) crystalline suspension in 3.2 M (NH$_4$)$_2$SO$_4$ solution (10 mg/mL) approx. 200 U/mg at 25 °C
VIII Lactate dehydrogenase (LDH) ; (from pig heart, Oriental Yeast Co. Ltd, Product Code: LDH-02) ammonium sulfate suspension, approx. 5,000 U/mL at 25 °C

Preparation of Enzyme Solution
Dissolve the lyophilized enzyme with distilled water and dilute to 1 to 5 U/mL with 50 mM Tris-HCl buffer, pH 8.5.

Procedure
(Reaction I)
1. Prepare the following reaction mixture and pipette 0.55 mL of reaction mixture into a test tube.
   Solution I 2.50mL Solution III 1.00mL
   Solution II 1.00mL H$_2$O 1.00mL
2. Add 0.10 mL of enzyme solution and mix.
3. Incubate at 60 °C for exactly 10 minutes.
4. After incubation, add 0.01 mL conc. HCl and mix.
5. Centrifuge at 10,000 rpm for 30 seconds.
   At the same time, repeat the Procedure 1 to 5 using distilled water in place of enzyme solution in Procedure 2 (as blank).

(Reaction II)
6. Prepare the following reaction mixture and pipette 2.50 mL of the reaction mixture into a cuvette.
   Solution IV 24.18mL Solution VII 0.12mL
7. Incubate at 30 °C for about 3 minutes.
8. Add 0.10 mL of supernatant of Procedure 5 and mix.
9. Read absorbance at 340 nm (Abs\text{*test}).
   Repeat the Procedure using blank (Abs\text{*blank}).

**Calculation**

\[
\text{Volume activity (U/mL)} = \frac{(\text{Abs}\text{*blank}) - (\text{Abs}\text{*test})}{6.22 \times 0.10 \times 0.10} \times \frac{2.60 \times 0.65}{60} \times \text{d.f.}
\]

\[
\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)}}
\]

\text{d.f.}; \text{dilution factor}

6.22; \text{millimolar extinction coefficient of NADH (cm}^2/\mu\text{mol)}

*\text{Protein concentration}; \text{determined by the absorbance at 280nm (Abs}_{280}), where 1 \text{Abs}_{280} = 1 \text{mg/mL}

**REFERENCES**

Fig. 1  pH profile
- ■ Tris-HCl

Fig. 2  pH stability
- ■ Tris-HCl, ■ Gly-KCl-KOH
treated for 24 hr at 4 °C in the following buffer solution (0.1 M):
- ● Tris-HCl, ■ Gly-KCl-KOH

Fig. 3  Thermal stability
- ○ Tris-HCl treated for 15 min in 0.1 M Tris-HCl buffer, pH 8.5

Fig. 4  Thermal stability
- ● Tris-HCl, ■ Gly-KCl-KOH
treated in 0.1 M Tris-HCl buffer, pH 8.5
- ○ 55 °C, ■ 60 °C, ● 65 °C
Fig. 5  Effect of various cations on the activity of Polynucleotide phosphorylase in the following Assay Method

Measurement: 0.015 mL of each cation solution, 0.010 mL of enzyme solution and 0.055 mL of reaction mixture were mixed, and reacted at 60 °C. After 10 minutes, the quantity of ADP was determined.

○ NaCl, △ KCl, □ MgCl$_2$, ● CaCl$_2$, ▲ ZnCl$_2$

Fig. 6  Effect of various anions on the activity of Polynucleotide phosphorylase in the following Assay Method

Measurement: 0.015 mL of each anion solution, 0.010 mL of enzyme solution and 0.055 mL of reaction mixture were mixed, and reacted at 60 °C. After 10 minutes, the quantity of ADP was determined.

○ NaCl, △ CH$_3$COONa, □ Na$_2$SO$_4$, ● NaHCO$_3$, ▲ NaH$_2$PO$_4$