Alkaline Phosphatase (ALP)

from calf intestinal mucosa

Orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1

An orthophosphoric monoester + H_2O ⟶ An alcohol + Orthophosphate

SPECIFICATION

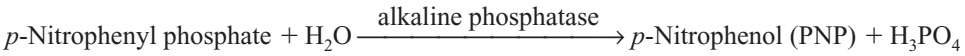
Appearance	liquid form (50% glycerol solution)
Activity	≧6,000 U/mg protein
Storage	at 4°C (do not freeze)

PROPERTIES

Molecular weight	*ca*. 140 kDa (gel filtration)	
pH Optimum	9.5–10.0	(Fig. 1)
pH Stability	6.5–11.0	(Fig. 2)
Optimum temperature	50°C	(Fig. 3)
Thermal stability	below *ca*. 40°C	(Fig. 4)
Stability (liquid form)	stable at 37°C for at least four weeks	(Fig. 5)
Stability (liquid form)	stable at 5°C for at least two years	(Fig. 6)
Activators	Mg^{2+}, Tris buffer, diethanolamine	
Inhibitors	inorganic phosphate, EDTA	

ASSAY PROCEDURE

Principle



The appearance of *p*-nitrophenol is measured spectrophotometrically at 405 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of PNP per min at 37°C and pH 9.8 under the conditions described below.

Reagents

A. $MgCl_2$ solution, 1.0 M: 2.03 g of $MgCl_2 \cdot 6H_2O$/10 ml of distilled water.
B. Diethanolamine buffer, 1.0 M; pH 9.8, containing 0.5 mM $MgCl_2$: dissolve 52.9 g of diethanolamine in 400 ml of distilled water and add 0.25 ml of $MgCl_2$ solution (Reagent A). Heat the solution to 37°C, then adjust to pH 9.8 with 2 N HCl and dilute with distilled water to 500 ml. Store in a dark bottle and prepare freshly before measurement.
C. *p*-Nitrophenyl phosphate solution, 0.65 M: 247 mg of *p*-nitrophenyl phosphate/1.0 ml of distilled water. Store in a dark bottle and prepare freshly before measurement.

Sample: dilute the enzyme preparation to a volume activity of 0.10–0.20 U/ml in ice-cold diethanolamine buffer (Reagent B) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).
   2.90 ml Diethanolamine buffer (Reagent B)
   0.05 ml *p*-Nitrophenyl phosphate solution (Reagent C)
2. Equilibrate at 37°C for about 5 min.
3. Add 0.05 ml of sample and mix.
4. Record the increase of absorbance at 405 nm in a spectrophotometer thermostated at 37°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_S).
   The blank solution is prepared by adding diethanolamine buffer (Reagent B) instead of sample (ΔA_0).

Calculation

Activity can be calculated using the following formula:

$$\text{Volume activity (U / ml)} = \frac{(\Delta A_S - \Delta A_0) \times 3.0\,(\text{ml}) \times df}{18.2 \times 0.05\,(\text{ml})} = \Delta A \times 3.30 \times df$$

18.2 : Millimolar extinction coefficient of PNP under the assay conditions ($cm^2/\mu mol$)
df : Dilution factor

APPLICATIONS

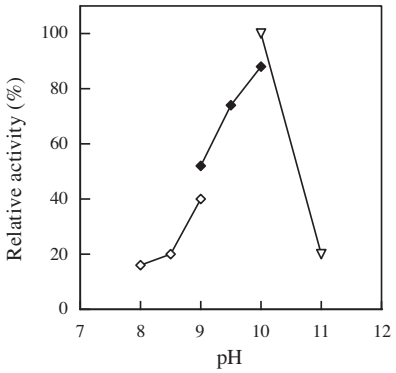
The enzyme is widely used as a marker in ALP based immunoassay.

REFERENCES

O'Sullivan, M. J. *et al*., *Anal. Biochem*., **100**, 100–108 (1979).
Bergmeyer, H. U., "Methods of Enzymatic Analysis," Vol. 2 (3rd ed.), Verlag Chemie, Weinheim, Germany, 1983, pp. 269–270.
Crowther, J. R., "ELISA: Theory and Practice," Humana Press, New York, 1995.
Price, C. P. and Newman, D. J., "Principles and Practice of Immunoassay," Macmillan Press, London, 1997.

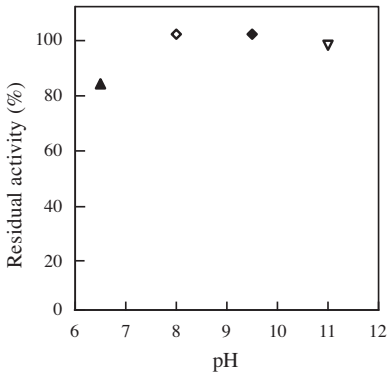
EXPERIMENTAL DATA

Fig. 1 pH Optimum



◇: 0.1 M TAPS-NaOH buffer
◆: 0.1 M CHES-NaOH buffer
▽: 0.1 M CAPS-NaOH buffer

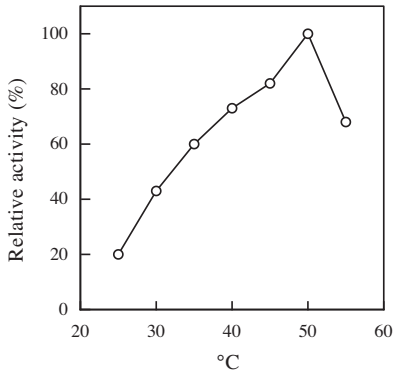
Fig. 2 pH Stability



Treatment: 25°C, 25 h

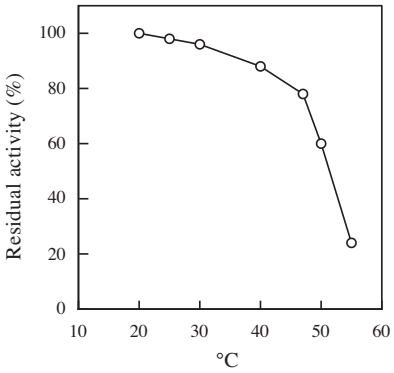
▲: 0.1 M MOPSO-NaOH buffer
◇: 0.1 M TAPS-NaOH buffer
◆: 0.1 M CHES-NaOH buffer
▽: 0.1 M CAPS-NaOH buffer

Fig. 3 Optimum temperature



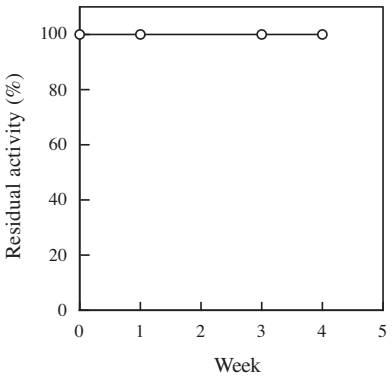
Buffer: 25 mM glycine-NaOH buffer, pH 9.6

Fig. 4 Thermal stability



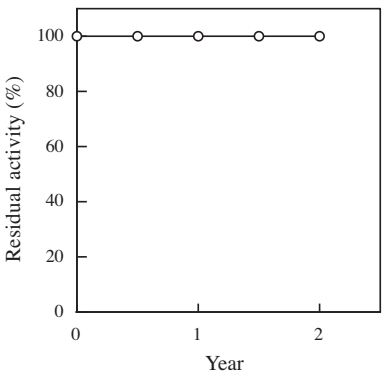
Treatment: 25 mM glycine-NaOH buffer, pH 9.6, containing 1 mM $MgCl_2$ and 0.1 mM $ZnCl_2$, 30 min

Fig. 5 Stability (liquid form) at 37°C



[Kept in 5 mM Tris-HCl buffer, pH 7.0, containing 50% glycerol, 5 mM $MgCl_2$ and 0.1 mM $ZnCl_2$]

Fig. 6 Stability (liquid form) at 5°C



[Kept in 5 mM Tris-HCl buffer, pH 7.0, containing 50% glycerol, 5 mM $MgCl_2$ and 0.1 mM $ZnCl_2$]