(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T-08

ALKALINE PHOSPHATASE [ALP]

from *Escherichia coli*
(Orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1)

Orthophosphoric monoester + H_2O ⟶ Alcohol + H_3PO_4

Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 40 U/mg solid

Properties

Molecular weight	: 80 kDa (Sephadex G-200)	
Isoelectric point	: pH 4.5	
Optimum pH	: 9.0	Figure 1
pH stability	: 8.5-10.0 (37℃, 60 min)	Figure 2
Thermal stability	: Stable at 45℃ and below (pH 9.0, 10 min)	Figure 3
Storage stability	: At least one year at −20℃	Figure 4
Activators	: Na^+, Mg^{2+}	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **lecithin** when coupled with phospholipase C (T-11) and choline oxidase (T-05).

$$\text{Lecithin} + H_2O \xrightarrow{\text{PLC}} \text{Phosphorylcholine} + \text{Diglyceride}$$

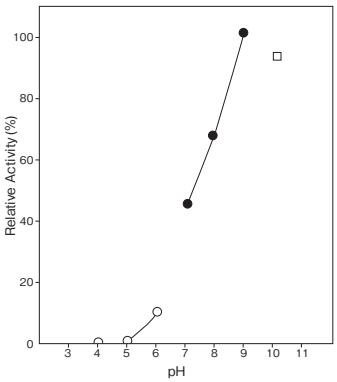
$$\text{Phosphorylcholine} + H_2O \xrightarrow{\textbf{ALP}} \text{Choline} + \text{Pi}$$

$$\text{Choline} + 2\,O_2 + H_2O \xrightarrow{\text{COD}} \text{Betaine} + 2\,H_2O_2$$

$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

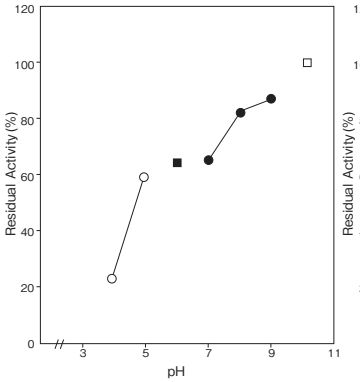
A

Fig.1 pH Optimum



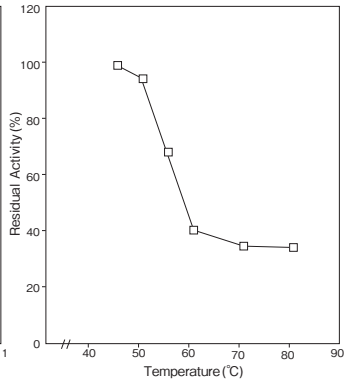
○ : Acetate buffer
● : Tris-HCl buffer
□ : Glycine-NaOH buffer

Fig.2 pH Stability



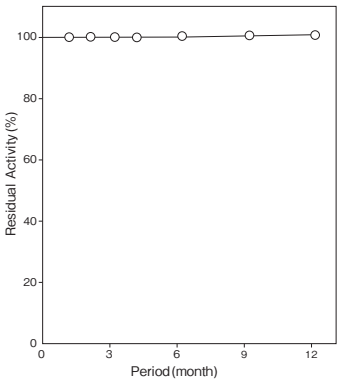
37℃,60 min
○ : Acetate buffer
■ : Phosphate buffer
● : Tris-HCl buffer
□ : Glycine-NaOH buffer

Fig.3 Thermal Stability



pH 9.0,10 min.
10 mM Tris-HCl

Fig.4 Storage (lyophilized powder)



−20℃

Assay

■ Principle

The assay is based on the increase in absorbance at 420 nm as *p*-nitrophenol is liberated according to the following reaction:

$$p\text{-Nitrophenylphosphate} + H_2O \xrightarrow{ALP} p\text{-Nitrophenol} + \text{Orthophosphate}$$

■ Unit definition

One unit is defined as the amount of enzyme which liberates 1 μmole of *p*-nitrophenol per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

0.5 M Tris-HCl buffer pH 9.0	0.20 ml
10 mM p-nitrophenylphosphate solution	0.20 ml
4 M NaCl solution	1.00 ml
Distilled water	0.50 ml

2. Reaction stopper
   0.5 N NaOH solution
3. Enzyme dilution buffer
   50 mM Tris-HCl buffer pH 9.0
4. Reagents
   p-nitrophenylphosphate ($2Na \cdot 6H_2O$):
   FUJIFILM Wako Pure Chemical Corporation #149-02342

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 1.90 ml of reaction mixture into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 100 μl of enzyme solution and mix to start the reaction at 37℃.

※ In the case of a test blank, add 100 μl of enzyme dilution buffer in place of enzyme solution.

3. At 10 minutes after starting the reaction, add 1.0 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 420 nm.

Absorbance sample : As
blank : Ab
$\triangle A = (As - Ab) \leqq 0.50$ Abs

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/10}{14.1} \times \frac{3.00}{0.10} \times \frac{1}{X}$$

14.1 : millimolar extinction coefficient of *p*-nitrophenol at 420 nm (cm^2/ μmole)
10 : reaction time (min)
3.00 : final volume (ml)
0.10 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at -20℃ in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 4).

References

1. Malamy, M. and Horecker, B.L. (1966) Methods Enzymol., **9**, 639.
2. Rotman, F. and Byrne, R. (1963) J. Molec. Biol., **6**, 330.
3. Garen, A. and Levinthal, C. (1960) Biochim. Biophys. Acta, **38**, 470.
4. Heppel, L. A., Harkness, D. R. and Hilmoe, R. J. (1962) J. Biol. Chem., **237**, 841.
5. Dray F., Dith E. and Rougeot C. (1986) Method of Enzymatic Analysis, Vol. 9, 348-362.
6. Rathman P. and Saxena B. B. (1986) Methods of Enzymatic Analysis, Vol. 9, 396-404.

ALP 活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液

0.5M トリス－HCl 緩衝液 pH9.0	0.20 ml
10mM *p*- ニトロフェニルリン酸溶液	0.20 ml
4M NaCl 溶液	1.00 ml
精製水	0.50 ml

2. 反応停止液
   0.5N NaOH 液
3. 酵素溶解希釈用液
   50mM トリス－HCl 緩衝液 pH9.0
4. 試薬
   p- ニトロフェニルリン酸･2Na･6H_2O：
   富士フイルム和光純薬製　特級　#149-02342

Ⅱ．酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 1.90ml を正確に分注して 37℃で予備加温する。
2. 5 分経過後、酵素試料液 100 μl を正確に加えて混和し、37℃で反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 100 μl を加える。
3. 10 分経過後、反応停止液 1.0ml を正確に加えて混和し、反応を停止する。
4. 420nm における吸光度を測定する。
   求められた吸光度を試料液は As、盲検液は Ab とする。

$$\Delta A = (As - Ab) \leqq 0.50\ Abs$$

Ⅳ．計算

$$活性\ (U/mg) = \frac{\Delta A/10}{14.1} \times \frac{3.00}{0.10} \times \frac{1}{X}$$

14.1 : *p*- ニトロフェノールの 420nm におけるミリモル分子吸光係数　（$cm^2/\ \mu mole$）
10　: 反応時間（min）
3.00 : 反応総液量（ml）
0.10 : 反応に供した酵素試料液量（ml）
X　: 酵素試料液中の検品濃度（mg/ml）