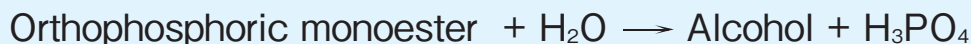


ALKALINE PHOSPHATASE [ALP]

from *Escherichia coli*

(Orthophosphoric-monoester phosphohydrolase (alkaline optimum) , EC 3.1.3.1)



Preparation and Specification

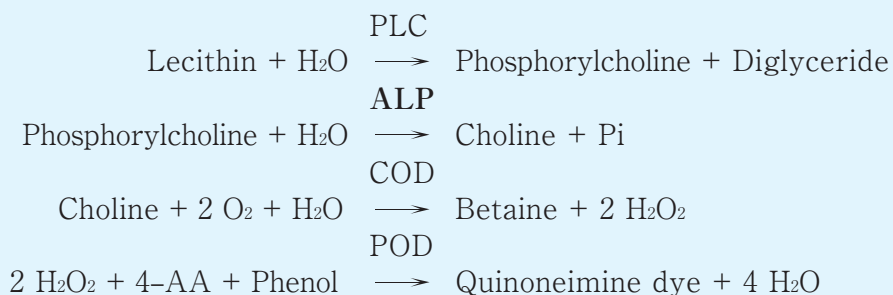
Appearance : White amorphous powder, lyophilized
 Specific activity : More than 40 U/mg solid

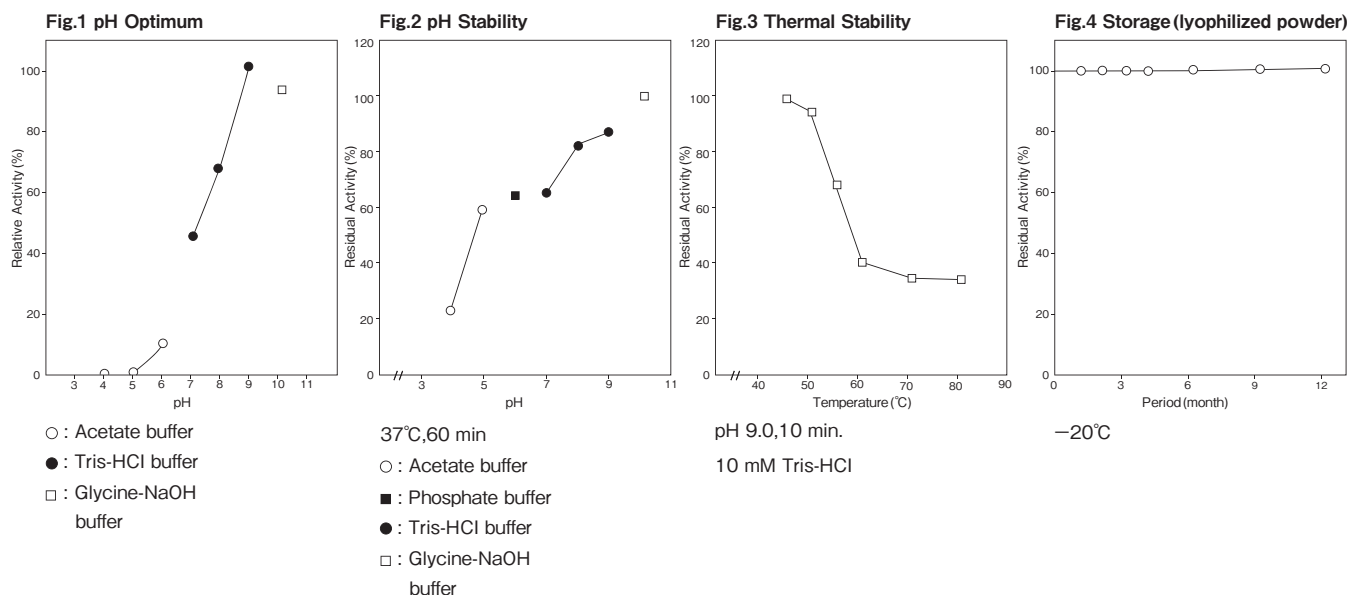
Properties

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°C, 60 min)	Figure 2
Thermal stability	: Stable at 45°C and below (pH 9.0, 10 min)	Figure 3
Storage stability	: At least one year at –20°C	Figure 4
Activators	: Na ⁺ , Mg ²⁺	
Inhibitor	: Orthophosphate	

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) .

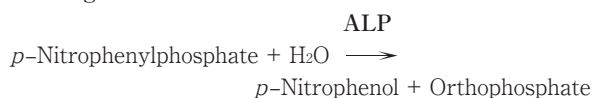




Assay

Principle

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



Unit definition

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

Reagents

- Reaction mixture

0.5 M Tris-HCl buffer pH 9.0	0.20 ml
10 mM <i>p</i> -nitrophenylphosphate solution	0.20 ml
4 M NaCl solution	1.00 ml
Distilled water	0.50 ml
- Reaction stopper
0.5 N NaOH solution
- Enzyme dilution buffer
50 mM Tris-HCl buffer pH 9.0
- Reagents
p-nitrophenylphosphate (2Na·6H₂O):
Wako Pure Chemical Industries, Ltd. # 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

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

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

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

$$\begin{aligned} \text{Absorbance sample} &: A_s \\ \text{Absorbance blank} &: A_b \\ \Delta A &= (A_s - A_b) \leq 0.50 \text{ Abs} \end{aligned}$$

Calculation

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

- 14.1 : millimolar extinction coefficient of *p*-nitrophenol at 420 nm (cm²/ 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°C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 4).

References

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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 <i>p</i> -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・6H₂O:
和光純薬工業製 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。

$$\Delta A = (A_s - A_b) \leq 0.50 \text{ Abs}$$

IV. 計算

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

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 (cm²/ μmole)

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)