TOYOBO ENZYMES

(Diagnostic Reagent Grade)

ALKALINE PHOSPHATASE

(Code : LPP-229)

from Microorganism

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

Orthophosphoric monoester + H_2O ⟶ Alcohol + Orthophosphate

PREPARATION and SPECIFICATION

Appearance	: Transparent liquid	
Activity	: Grade II 30,000 U/ml or more	
Contaminants	: Adenosine deaminase	$\leq 1.0\times10^{-4}$%
	Phosphodiesterase	$\leq 3.0\times10^{-3}$%

PROPERTIES

Stability	: Stable at 4°C	
Molecular weight	: approx. 104,000	
Optimum pH	: 9.5	(Fig. 1)
Optimum temperature	: > 60°C	(Fig. 2)
pH Stability	: pH 5.5-10.4 (25°C, 16hr)	(Fig. 3)
Thermal stability	: below 65°C (pH 7.0, 60min)	(Fig. 4)

APPLICATIONS

This enzyme is useful for molecular biology.

ASSAY

Principle :

p-Nitrophenylphosphate(pNPP) + H_2O $\xrightarrow{\text{alkaline phosphatase}}$ p-Nitrophenol + Pi

The appearance of p-Nitrophenol is measured at 405nm by spectrophotometry.

Unit definition :

One unit causes the formation of one micromole of p-Nitrophenol per minute under the conditions described below.

Method :

Reagents

A. Diethanolamine buffer, : 1M [Dilute 9.66ml of diethanolamine (MW=105.14) in 60ml of H_2O, add 5ml of 0.1M $MgCl_2$ and, after adjusting the pH to 9.8 with 2N HCl, fill up to 100ml with H_2O] (Prepare fleshly)

B. pNPP solution : 0.674M [2.5g of p-Nitrophenylphosphate disodium salt (MW=371.16) / 10ml Diethanolamine buffer (A)] (Prepare fleshly)

C. Enzyme diluent : 30mM Triethanolamine, 1mM $MgCl_2$, 0.1mM $ZnCl_2$, 0.1% Triton X-100, pH7.6

Procedure

1. Prepare the following working solution (30.5ml) in a brownish bottle and store on ice (Prepare freshly)

30ml	Diethanolamine buffer	(A)
0.5ml	pNPP solution	(B)

Concentration in assay mixture	
Diethanolamine	0.97 M
p-Nitrophenylphosphate	11 mM
$MgCl_2$	4.8 mM

2. Pipette 3.0ml of working solution into a cuvette (d=1.0cm) and equilibrate at 37°C for about 5 minutes.
3. Add 0.1ml of the enzyme solution* and mix by gentle inversion.
4. Record the increase in optical density at 405nm against water for 3 to 5 minutes in a spectrophotometer thermostated at 37°C, and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test). At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent (C) is added instead of the enzyme solution.

*Dilute the enzyme preparation to 0.1-0.3U/ml with ice-cold enzyme diluent (C), immediately before assay.

Calculation :

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{\Delta OD/min(\Delta OD\ test - \Delta OD\ blank) \times Vt \times df}{1.85 \times 1.0 \times Vs} = \Delta OD/min \times 1.676 \times df$$

Weight activity (U/mg) = (U/ml)×1/C

Vt : Total volume (3.1ml)

Vs : Sample volume (0.1ml)

18.5 : Millimolar extinction coefficient of p-Nitrophenol under the assay condition (cm^2/micromole)

1.0 : Light path length (cm)

df : Dilution factor

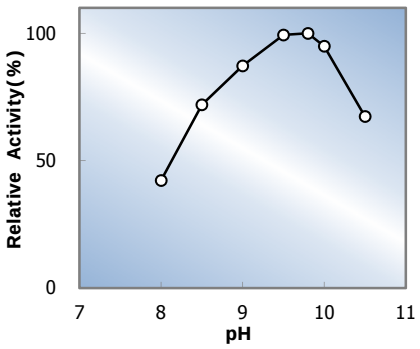

Fig.1 pH-Activity

in 1M Diethanolamine buffer, pH 8-10.5

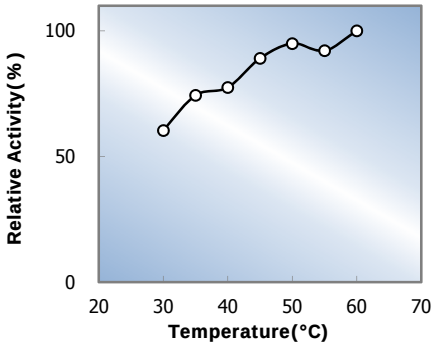

Fig.2 Temperature activity

in 1M Diethanolamine buffer, pH 10.25

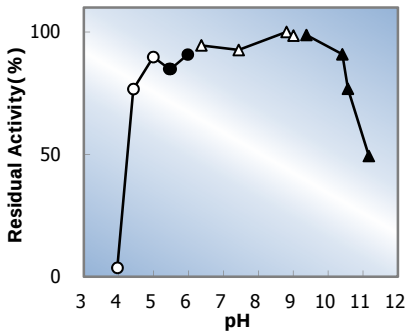

Fig.3 pH-Stability

25°C, 16hr-treatment with 0.1M buffer solution: pH 4-6, dimethylglutaric acid-NaOH; pH 6-8, K-phosphate; pH 8-9, Tris-HCl; pH 9-10, glycine-NaOH.
Enzyme concentration: 10U/ml

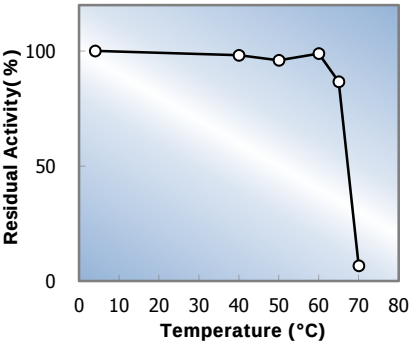

Fig.4 Thermal stability

15min-treatment with 50mM K-phosphate buffer, pH 7.0.
Enzyme concentration: 10U/ml

活性測定法 (Japanese)

1. 原理

$$\text{p-Nitrophenylphosphate(pNPP)} + H_2O \xrightarrow{\text{alkaline phosphatase}} \text{p-Nitrophenol} + Pi$$

p-Nitrophenolの生成量を405nmにおける吸光度の変化で測定する。

2. 定義

下記条件下で1分間に1マイクロモルのp-Nitrophenolを生成する酵素量を1単位(U)とする。

3. 試薬

A. 1Mジエタノールアミン緩衝液、pH9.8 [9.66mlのジエタノールアミン (MW=105.14)を蒸留水60mlで希釈後、0.1M $MgCl_2$ 5mlを添加する。さらに2N HClで37°CにおけるpHを9.8に調整し、最終液量を100mlとする] (用時調製)
B. 0.674M pNPP溶液 [2.5gのp-ニトロフェニルリン酸二ナトリウム塩 (MW=371.16)を10mlの緩衝液Aに溶解する] (用時調製)
C. 酵素溶液: 30mM トリエタノールアミン、1mM MgCl2、0.1mM ZnCl2、0.1% Triton X-100, pH7.6で反応直前に0.1～0.3U/mlに希釈する。

4. 手順

① 下記反応混液(30.5ml)を調製する。

30ml ジエタノールアミン緩衝液	(A)
0.5ml pNPP溶液	(B)

② 3.0mlの反応混液をキュベット(d=1.0cm)に移し、37°Cで約5分間予備加温する。

③ 酵素溶液0.1mlを添加し、ゆるやかに混和する。

④ 水を対照に37°Cに制御された分光光度計で405nmの吸光度変化を3~5分間記録し、その初期直線部分から1分間当たりの吸光度変化を求める(ΔOD test)。盲検は酵素溶液に代えて酵素希釈液を加え、上記同様に操作を行って1分間当たりの吸光度変化を求める(ΔOD blank)。

5. 計算式

$$U/ml = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.00(ml) \times 希釈倍率}{18.5 \times 1.0 \times 0.1\ (ml)}$$

$$= \Delta OD/min \times 1.676 \times 希釈倍率$$

Vt ：総液量 (3.1ml)
Vs ：試料総量 (0.1ml)
18.5：p-Nitrophenolのミリモル分子吸光係数
1.0 ：光路長 (cm)