●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

ALKALINE PHOSPHATASE

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 30,000U/ml or more
Contaminants	: Adenosine deaminase ≤1.0×10^{-4}%
	Phosphodiesterase ≤3.0×10^{-3}%

PROPERTIES

Stability	: Stable at 4℃	
Molecular weight	: approx. 100,000	
Optimum pH	: 9.8	(Fig.1)
Optimum temperature	: ≧ 60℃	(Fig.2)
pH Stability	: pH 6.0－10.4 (25℃, 16hr)	(Fig.3)
Thermal stability	: below 60℃ (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, pH10.25 : 5.0mM［Dilute 9.66ml of diethanolamine (MW＝105.14) in 60ml of H_2O, add 0.25ml of 0.1M $MgCl_2$ and, after adjusting the pH to 10.25 with 2N HCl, fill up to 100ml with H_2O］(Prepare fleshly)

B. pNPP : 0.1M［Dissolve 371mg of p-nitrophenylphosphate disodium salt (MW＝371.16) in 10ml buffer solution A］(Prepare fleshly)

C. Enzyme diluent : 0.1M Diethanolamine buffer pH 9.5 contg. 0.25mM $MgCl_2$

Procedure

1. Prepare the following reaction mixture in a cuvette (d＝1.0cm) and equilibrate at 37℃ for about 5 minutes.

2.6ml	Buffer solution	(A)
0.3ml	Substrate solution	(B)

Concentration in assay mixture	
Diethanolamine buffer	0.97 M
p-Nitrophenylphosphate	10 mM
$MgCl_2$	0.25mM

2. Add 0.1ml of the enzyme solution* and mix by gentle inversion.
3. Record the increase in optical density at 405nm against water for 3 to 5 minutes in a spectrophotometer thermostated at 37℃, 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 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 :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min}\,(\Delta\text{OD test} - \Delta\text{OD blank}) \times \text{Vt} \times \text{df}}{18.5 \times 1.0 \times \text{Vs}} = \Delta\text{OD/min} \times 1.62 \times \text{df}$$

Vt : Total volume (3.0ml)

Vs : Sample volume (0.1ml)

18.5 : Millimolar extinction coefficient of p-Nitrophenol under the assay condition (㎤/micromole)

1.0 : Light path length (cm)

df : Dilution factor

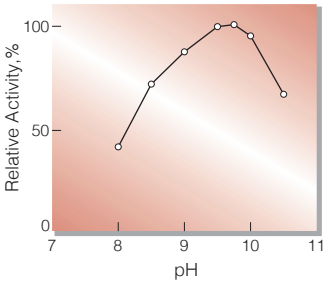

Fig.1. pH-Activity

〔in 1M Diethanolamine buffer, pH 8-10.5〕

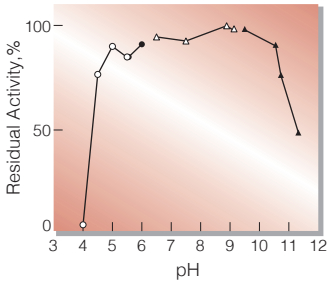

Fig.3. pH-Stability

〔25℃, 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〕

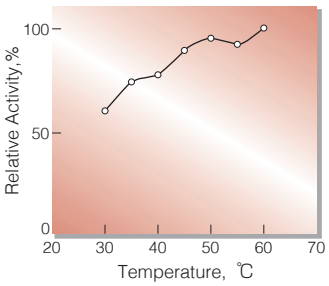

Fig.2. Temperature activity

〔in 1M Diethanolamine buffer, pH 10.25〕

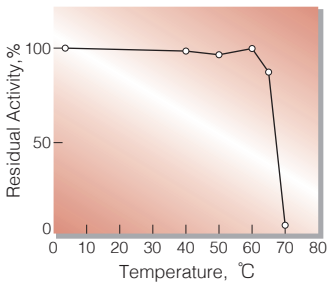

Fig.4. Thermal stability

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

活性測定法（Japanese）

1.原理

p-Nitrophenylphosphate(pNPP)＋H_2O
$\xrightarrow{\text{alkaline phosphatase}}$ 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，pH10.25〔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 $MgCl_2$ 0.25mℓを添加する。さらに，2.0N HClでpHを10.25に調整し，最終液量を100mℓとする〕(用時調製)

B. 0.1M pNPP溶液〔371mg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)

酵素溶液：酵素溶液を予め氷冷した0.25mM $MgCl_2$を含む0.1Mジエタノールアミン緩衝液,pH 9.5で分析直前に0.1～0.3/mℓに希釈する。

4.手順

①下記反応混液をキュベット(d＝1.0cm)に調製し，37℃で約5分間予備加温する。

2.6mℓ	ジエタノールアミン緩衝液	(A)
0.3mℓ	基質溶液	(B)

②酵素溶液0.1mℓを添加し，ゆるやかに混和後，水を対照に37℃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分間当りの吸光度変化を求める(ΔODtest)。

③盲検は反応混液①に酵素溶液の代りに酵素希釈液(0.25mM $MgCl_2$を含む0.1Mジエタノールアミン緩衝液，pH9.5)を加え，上記同様に操作を行って1分間当りの吸光度を求める(ΔODblank)。

5.計算式

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

$$= \Delta OD/min \times 1.62 \times 希釈倍率$$

18.5 ：p-Nitrophenolのミリモル分子吸光係数
1.0 ：光路長(cm)