●TOYOBO ENZYMES●

(Industrial Grade)

IMMOBILIZED LIPASE

from Pseudomonas sp.

PREPARATION and SPECIFICATION

The enzyme is a preparation of a *Pseudomonas* sp. lipase (TOYOBO lipoprotein lipase Grade Ⅲ LPL-311) immobilized on Hyflo Super-Cel.

Appearance	: Light brown powder (immobilized on Hyflo Super-Cel)
Activity	: 0.5U/mg-solid or more
Stabilizers	: Sugars

APPLICATIONS [3,11]

This enzyme is useful for enzymatic ester synthesis, transesterification, acidolysis and alcholysis in organic solvents or solvent free substrate.

REFERENCES

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2) *Agric.Biol.Chem.,* 53 (7), 1879－1884 (1989)
3) *J.Bull.Inst.Chem.Res., Kyoto Univ.* 70 (3), 333－337 (1992)
4) *Tetrahedron Letters, 31, No.25,* 3603－3604 (1990)
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6) *Tetrahedron Letters, 35 No.43,* 7997－8000 (1994)
7) *Tetrahedron:Asymmetry 6, No.7,* 1617－1620 (1995)
8) *Tetrahedron 51 No.34,* 9339－9352 (1995)
9) *Biosci. Biotech. Biochem., 59 (11),* 2178－2180 (1995)
10) *Macromolecules 28, No.21,* 7046－7050 (1995)
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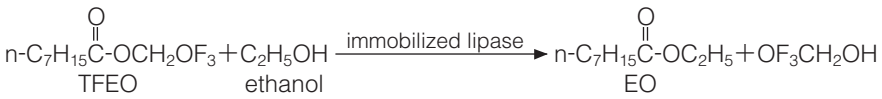
ASSAY

Principle:



Unit definition:

One unit causes the formation of the one micromole of ethyloctanoate (EO) per minute under the conditions described below.

Procedure

1. Pipette 10.0ml of dry diisopropyl ether containing 50mM 2,2,2-trifluoroetyl octanoate (TFEO) and 0.2M ethanol into a test tube with a magnetic stirrer bar and a stopper. Equilibrate at 25.5℃ for about 10min with stirring.
2. Add about 20mg of immobilized lipase powder and stir constantly.
3. Take an aliquot of the assay mixture at a specific time interval and analyse with capillary gas chromatography (DB-5, J&W Scientific, 160℃). The retention time of TFEO and the product EO are 2.14 and 2.54 min, respectively.
4. A plot natural logarithm of the reaction conversion (C) vs reaction time gives a straight line $[-\ln(1-C)=k_0 t]$, and the initial rate (V_0) of the reaction is calculated from the slope of the line.

Calculation

Activity can be calculated by using the following formula：

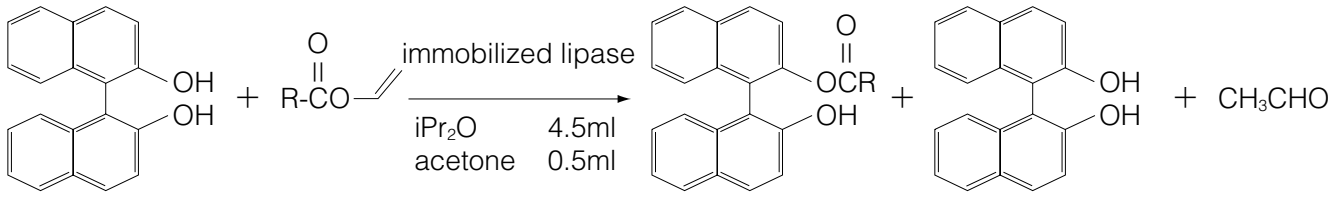
Initial rate (V_0) $= k_0 \times 50$

Weight activity (U/mg) $= \frac{V_0 \times 10}{Ws}$

k_0 ：Slope of the line $[-\ln(1-C)=k_0 t]$
50 ：Concentration of TFEO (mM)
Ws ：Sample weight (mg)
10 ：Total volume (ml)

Illustrations of Stereoselective Acylation

Scheme1. Stereoselective Acylation of Binaphthol [2)]

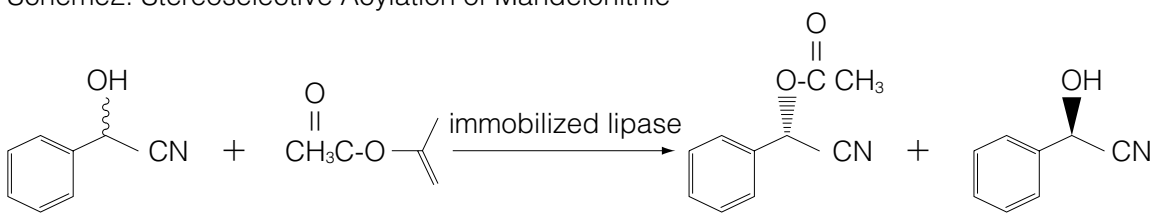


(±)−2　　1　　40℃　　(R)−(+)−3　　(S)−(−)−2

1: a R= CH_3
b nC_3H_7
c nC_5H_{11}
d nC_7H_{15}

2: binaphthol
3: binaphthol monoesters

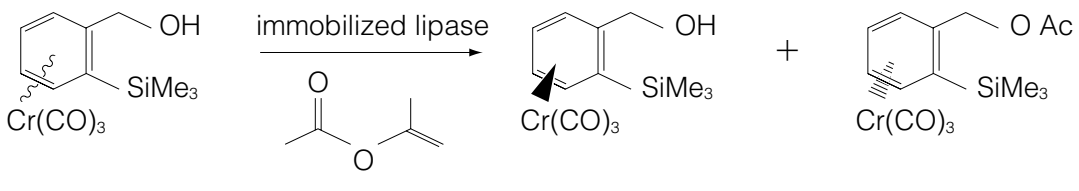
Scheme2. Stereoselective Acylation of Mandelonitrile [3)]



(±)−1　　3　　(S)−(−)−2　　(R)−(+)−1

1: 1-cyano-1-phenylmethanol (mandelonitrile)
2: 1-cyano-1-phenylmethanol acetate
3: isopropenyl acetate

Scheme3. Stereoselective Acylation of Tricarbonyl (o-trimethylsilylbenzyl alcohol) chromium [4)]

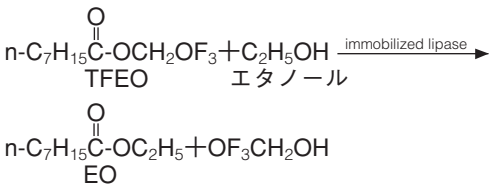


(R.S)1　　(R)1　　(S)2

1: tricarbonyl (o-trimethylsilylbenzyl alcohol) chromium
2: tricarbonyl (o-trimethylsilylbenzyl alcohol) chromium acetate

活性測定法（Japanese）

1.原理



2.定義

下記条件下で求めた,反応初期速度より算出した1分間に 1マイクロモルのethyloctanoate(EO)を生成する酵素活性を1Uとする。

3.手順

①50mM 2,2,2-trifluoroethyl octanoate (TFEO)及び,0.2Mエタノールを含む乾燥diisopropyl ether〔$(i\text{-}Pr)_2O$〕10mℓをスターラーバーの入った共栓付試験管に採り,攪拌しながら25.5℃で10分間予備加温する。

②固定化酵素　約20mgを上記反応液に添加し,1～20分間に数回サンプリングし,キャピラリーガスクロマトグラフィー (DB-5, J&W Scientific製,160℃)にて分析する。TFEO及びEOのretention timeは　約2.14及び2.54分である。

③EOへの反応の転換率(C)より,反応時間t(分)に対して−ln(1−C)をプロットし,その傾き(k_0)より下記計算式に従って初期速度(V_0)及び比活性(U/mg)を求める。

4.計算式

$V_0 = k_0 \times 50$

$U/mg = \frac{V_0 \times 10}{Ws}$

50　：　TFEO濃度 (mM)
K_0　：　速度定数
V_0　：　初期速度
U/mg　：　比活性
Ws　：　サンプル重量 (mg)