Strem Chemicals, Inc. www.strem.com

Catalog # 06-3123 Novozym® 435

Note: Sold in collaboration with Novozymes A/S for research purposes only.

Novozym® 435 is a CALB lipase immobilized on a hydrophobic carrier (acrylic resin). The product is a non-specific lipase originating from Candida Antarctica B. Key applications include the dynamic kinetic resolution of alcohols coupled with a ruthenium catalyst, and the dynamic kinetic resolution of an amine coupled with a metal racimizing agent.

Lipase CALB is stable over a relatively broad pH range, especially in the alkaline pH range. The enzyme exhibits a very high degree of substrate specificity, with respect to both regio- and enantioselectivity. Lipase CALB has been used extensively in the resolution of racemic alcohols, amines, acids, and in the preparation of optically active compounds from meso substrates. The resulting optically pure compounds are very hard to obtain by alternate routes and can be of great synthetic value. Likewise, CALB has been used intensively as a regio-selective catalyst in the selective acylation of different carbohydrates.

Declared activity 10000 PLU/g. Lipase that hydrolyzes ester bonds in glycerides. Color can vary from batch to batch. Color intensity is not an indication of enzyme activity. Packaging must be kept intact, dry and away from sunlight. Please follow the recommendations and use the product before the best before date to avoid the need for a higher dosage.

Advantages:

1. Mild and selective on multi-functional substrates.
2. Active for both bulk liquid substances and in the presence of organic co-solvents.
3. Performs well under anhydrous conditions and with moisture sensitive substrates.
4. Functions across a wide temperature range (20 – 110°)
5. Suitable for both stirred batch tank and continuous fixed bed reactors.
6. Can be recycled 5-10 times without activity loss, depending on reaction conditions.
7. Used in large scale industrial production.
8. Nil protein residues in final products.

Other Lipase products offered by Strem:

06-3118 Palatase® 20000 L
06-3155 Lipozyme® TL IM
06-3100 Novocor® AD L
06-3120 Lipozyme® RM
06-3105 Lipozyme® CALB L
06-3140 Lipozyme® TL 100 L
06-3125 Resinase® HT
06-3135 Novozym® 51032
96-0220 Novozymes Lipase Screening Kit (contains 9 lipase enzymes)

Endoprotease products offered by Strem:

06-3110 Alcalase® 2.4 L FG
06-3112 Alcalase® 2.5 L
06-3137 Savinase® 12 T
06-3150 Savinase® 16 L
06-3115 Esperase® 8.0 L
06-3160 Neutrase® 0.8 L
96-0224 Novozymes Endoprotease Screening Kit (contains 6 endoprotease enzymes)

Novozymes Lipase Products

Storage

Kits should be optimally stored at 0-10°C/32-50°F. If stored above 25°C/77°F the samples should be used within 3 months.

Introduction

Lipases (EC Number 3.1.1.3) are one of the most commonly used classes of enzymes in biocatalysis. They have been used on a variety of substrates and show very broad substrate specificity. Lipases catalyze the hydrolysis of triacylglycerols to diacylglycerol, monoacylglycerol, glycerol and free fatty acids. The reaction reverses under anhydrous conditions and the enzyme is able to synthesize new molecules by esterification, alcoholysis and transesterification. All reactions can be performed with high regio- and enantioselectivity under mild reaction conditions.

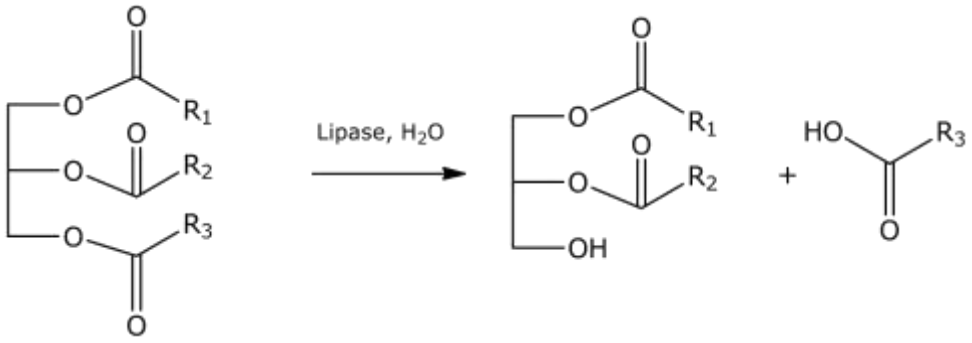


Figure 1: Regioselective hydrolysis of a triacylglycerol.

Description and optimum usage conditions

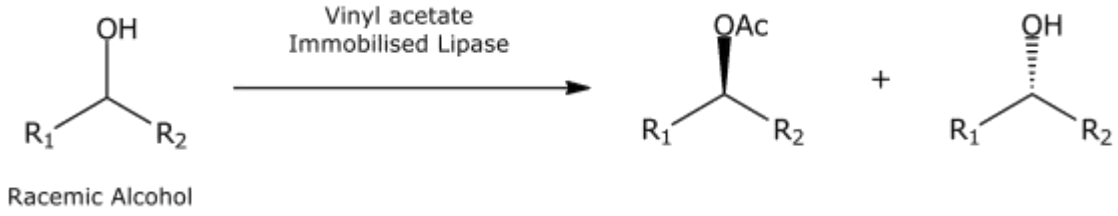
Enzyme No.	Strem Catalog Number	Product Name	Activity*	Formulation	pH optimum	Temp optimum	Substrate specificity
1.	**06-3123**	Novozym® 435	10000 PLU/g	Immobilized	pH 5-9	30-60°C	Esters and alcohols
2.	**06-3155**	Lipozyme® TL IM	250 IUN/g	Immobilized	pH 6-8	50-75°C	Esters
3.	**06-3120**	Lipozyme® RM	275 IUN/g	Immobilized	pH 7-10	30-50°C	Esters
4.	**06-3105**	Lipozyme® CALB L	5000 LU/g	Liquid	pH 5-9	30-60°C	Esters and alcohols
5.	**06-3118**	Palatase® 20000 L	20000 LU/g	Liquid	pH 7-10	30-50°C	Esters
6.	**06-3140**	Lipozyme® TL 100 L	100 KLU/g	Liquid	pH 7-10	20-50°C	Esters and diesters
7.	**06-3100**	NovoCor® AD L	6000 LU/g	Liquid	pH 5-9	30-60°C	Sterically hindered esters
8.	**06-3125**	Resinase® HT	50 KLU/g	Liquid	pH 5-8	up to 90°C	Esters
9.	**06-3135**	Novozym® 51032	15 KLU/g	Liquid	pH 7-10	35-70°C	Esters

* K = Kilo, LU = Lipase unit, PLU = Propyl Laurate Unit, IUN = Interesterification Unit.

1LU is the amount of enzyme activity which liberates 1 µmol of tritratable butyric acid from the substrate glycerol tributyrate per minute under defined standard conditions. 1LU is equal to 1IUN. 1 PLU is the amount of enzyme activity which generates 1 µmol of propyl laurate per minute under defined standard conditions.

Kinetic Resolution

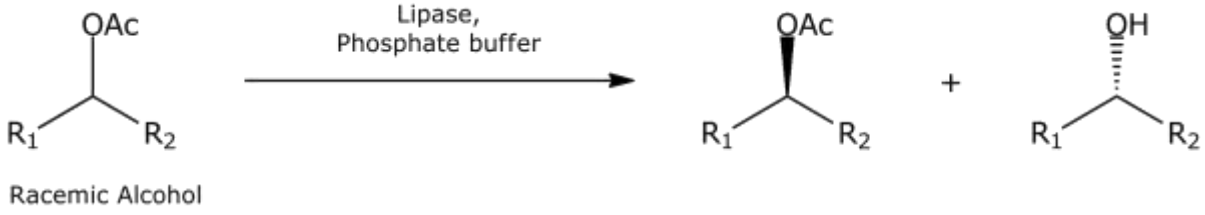
Example 1. Kinetic resolution by transesterification of racemic alcohol[1]



- Racemic alcohol (1-2 mmol) is solubilized in organic solvent (10 mL Toluene (dry) or other solvent)*
- Acyl donor vinyl acetate (1:3 or 1:5 molar ratio compared to racemic alcohol) is added.
- Immobilized Lipase Enzyme**1-3** (50% wt/wt with regards to substrate) is added and the reaction is conducted under stirring.
- Typical reaction temperature is 25-50^{o}C and typical reaction time is 36-72 hours, depending on substrate.
- The reaction product is recovered by removing the immobilized enzyme by filtration.

* Alternative solvents: methyl tert butyl ether (MTBE), n-hexane, iso-octane.

Example 2. Kinetic resolution of racemic alcohol by hydrolysis of acetoxy derivative

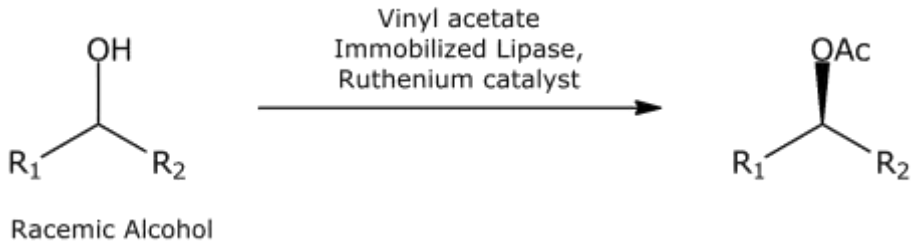


- Racemic acetoxy ester (1-2 mmol) is solubilized/dispersed in potassium phosphate buffer (0.1 M, pH 7.5, 10 mL). For liquid substrates, emulsion or suspension will be formed. For solid substrates, solution is prepared by adding 10% v/v of organic solvent*
- Lipase Enzyme **1-9** is added to the substrate solution (50% wt/wt for solid enzyme or 10-20% v/v with regards to buffer for liquid enzyme)

- Reaction mixture pH is maintained at 7.5 by adjusting with 1N NaOH.
- Typical reaction temperature is 25-40°C and typical reaction time is 24-48 hours.
- The reaction product is recovered by extraction or filtration.

*Solvent examples: IPA, acetone, tert-butanol, THF or acetonitrile

Example 3. Dynamic kinetic resolution of racemic alcohols[2, 3, 4]



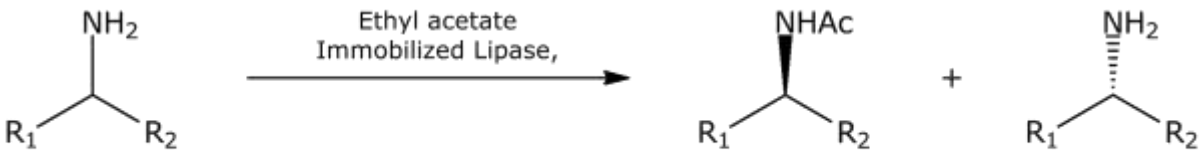
Racemic Alcohol

- Ruthenium catalyst* (0.05 eq with regards to substrate), Immobilized lipase **1-3** (50% w/w with regards to substrate) and Na_2CO_3 (1.0 eq. with regards to substrate) are dissolved in dry organic solvent (5 mL Toluene**) under inert atmosphere (N_2) in a closed vessel.
- Dry toluene is added and resulting mixture is stirred.
- A THF solution of t-BuOK (0.05 eq with regards to substrate) is added to reaction mixture.
- After 30 min. of stirring, racemic alcohol (1-2 mmol) dissolved in toluene (5 mL) is added at 25-30°C and stirring continued in 10 min.
- Vinyl acetate (3.0 eq with regards to substrate) is charged to reaction mixture at 25-30°C.
- Reaction temperature is 50 -55°C and typical reaction time is 36 hours.
- Reaction product is recovered by filtration through filter aid and the filtrate is concentrated to obtain crude product.

* Ruthenium catalyst options: Chlorodicarbonyl (1,2,3,4,5-pentaphenylcyclopentadienyl) ruthenium ($\eta^5C_5Ph_5$)$Ru(CO)_2Cl$ or Shvo catalyst - 1-Hydroxytetraphenylcyclopentadienyl-(tetraphenyl-2,4-cyclopentadien-1-one)-μ-hydrotetracarbonyldiruthenium(II)

** Solvents have to be dried before using (moisture content should be < 0.01%)

Example 4. Kinetic Resolution by transesterification of Racemic Amine[5, 6]



Racemic Alcohol

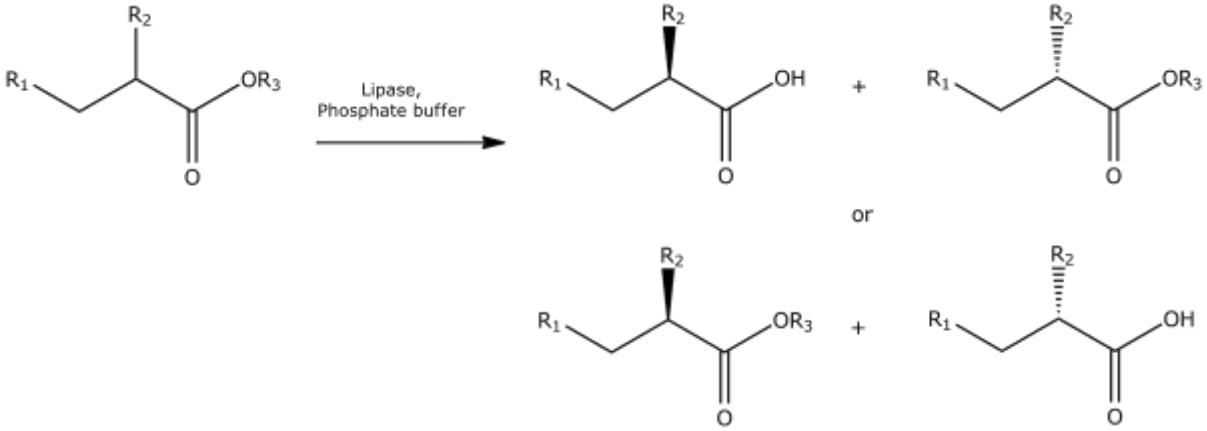
- Acyl donor* ethyl acetate (5 mL) is charged to vessel under inert atmosphere (N_2)
- Racemic amine (1-2 mmol) and Immobilized lipase **1-3** (50% wt/wt with regards to

substrate) is added to ethyl acetate at 25-50°C under moderate stirring and inert atmosphere.

- Typical reaction temperature is 25-40°C and typical reaction time is 36-72 hours, depending on substrate.
- Reaction product is recovered by filtration, whereby immobilized enzyme is removed.

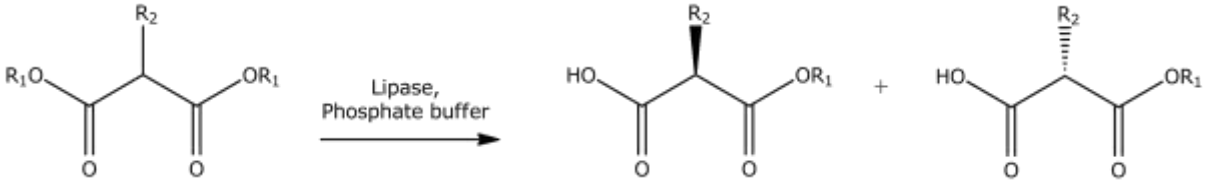
*Acyl donor solvent: α-methylbenzyl acetate, methylmethoxy acetate, ethyl acetate and methyl tert butyl ether.

Example 5. Kinetic resolution by hydrolysis of racemic carboxylic ester



- Racemic ester (1-2 mmol), organic solvent (5 mL MTBE or Toluene) and potassium phosphate buffer (0.1 M, pH 7.0, 5 mL) is homogenized by stirring. [Two layers will form once stirring is stopped; stir until substrate is soluble in organic phase. In case of immobilized enzymes, solid suspension is observed.]
- Lipase Enzyme (50% wt/wt with regards to substrate for solid enzyme **1-3** or 10-20% v/v with regards to solvent mixture for liquid enzyme **4-9**) is added under stirring.
- Reaction mixture is maintained at pH 7.0 by adjusting with 1N NaOH.
- Typical reaction temperature is 20-35°C and typical reaction time is 24-48 hours, depending on substrate.
- Reaction product is recovered by extraction or filtration.

Example 6. Desymmetrisation of diesters[7, 8]



- Racemic diester (1-2 mmol) and potassium phosphate buffer (0.1 M, pH 7.0, 10 mL) is homogenized by stirring.
    - For liquid substrates emulsion or suspension will be formed.
    - For solid substrates a solution is prepared by adding additional solvents such.
    - Biphasic reactions can be carried out by making solution in MTBE or toluene.
    - Solvent free reactions can be carried out in a solid suspension.

- Lipase Enzyme (50% wt/wt for solid enzyme **1-3** or 10-20% v/v with regards to buffer for liquid enzyme **4-9**) is added and stirring continued.
- Reaction mixture is maintained at pH 7.5 by adjusting with 1N NaOH.
- Typical reaction temperature is 25-40^{o}C and typical reaction time is 24-48 hours, depending on substrate.
- Reaction product is recovered by extraction or filtration.

*Solvent: acetone, tetrahydrofuran (THF) or acetonitrile.

Analytical Method Principles

In-process reaction monitoring:

Depending on substrate and product, different methods can be used for in process reaction monitoring.

- Thin Layer Chromatography (TLC) is a simple method for monitoring reaction progress and completion.
- To quantitatively estimate product formation and consumption of reactant, HPLC or GC can be used for monitoring.
- Chiral HPLC is recommended to estimate chiral purity or consumption of isomers of racemic mixture.
- Final chiral purity can be obtained by analyzing product isolated by using an appropriate chiral column.

Key parameters for Enantiomeric excess (ee) and Enantioselectivity (E) can be calculated from the areas in chiral HPLC:

% ee = ((R-S)/(R+S)) × 100 where R and S stand for the individual optical isomer in the mixture (and R +S = 1)
Where R = area for R isomer and S = area for S isomer

$$E = \frac{\ln\left[\frac{1 - e.e._s}{1 + e.e._s / e.e._p}\right]}{\ln\left[\frac{1 + e.e._s}{1 + e.e._s / e.e._p}\right]}$$

Screening Procedure

Listed below is recommended equipment for conducting the screens, however, pH-stat system gives more consistent results.

Simple equipment	Advanced equipment
Reaction vessel (25 mL round bottom or Erlenmeyer flask or test tubes)	Thermostated reaction vessel (25 mL)
pH-meter or pH-paper (range 5 - 9)	Autotitrator/pH-stat system (pH-meter, automatic burette/addition funnel)
Burette or calibrated addition funnel	Recording device (e.g., x/y-plotter)
Propeller mixer or magnetic needle	Propeller mixer

Buffer Preparation

0.1M Potassium Phosphate Buffer at 25°C		
pH	Volume of 1M K_2HPO_4 (mL)	Volume of 1M KH_2PO_4 (mL)
5.8	8.5	91.5
6.0	13.2	86.8
6.2	19.2	80.8
6.4	27.8	72.2
6.6	38.1	61.9
6.8	49.7	50.3
7.0	61.5	38.5
7.2	71.7	28.3
7.4	80.2	19.8
7.6	86.6	13.4
7.8	90.8	9.2
8.0	94.0	6.0
Dilute combined 1M stock solutions to 1 L with distilled H_2O.		

0.1M Sodium Phosphate Buffer at 25°C		
pH	Volume of 1M Na_2HPO_4(mL)	Volume of 1M NaH_2PO_4 (mL)
5.8	7.9	92.1
6.0	12.0	88.0
6.2	17.8	82.2
6.4	25.5	74.5
6.6	35.2	64.8
6.8	46.3	53.7
7.0	57.7	42.3
7.2	68.4	31.6
7.4	77.4	22.6
7.6	84.5	15.5
7.8	89.6	10.4
8.0	93.2	6.8
Dilute combined 1M stock solutions to 1 L with distilled H2O.		

References

1. H. V. Ferreira, L. C. Rocha, R.P. Severino and André L. M. Porto *Molecules* **2012**, *17*, 8955-8967
2. A.Traff, R. Lihammar, and J.E. Backvall J. Org. Chem. 2011, 76, 3917–3921
3. Mahn-Joo Kim, Yangsoo Ahn and Jaiwook Park *Current Opinion in Biotechnology* 2002, **13**:578–587
4. Kiwon Han, Cheolwoo Kim, Jaiwook Park and Mahn-Joo Kim *J. Org. Chem.* 2010, 75, 3105–3108
5. Javier Gonzalez-Sabın, Vicente Gotor and Francisca Rebolledo *Tetrahedron: Asymmetry* 16 (2005) 3070–3076
6. Mahn-Joo Kim, Won-Hee Kim, Kiwon Han, Yoon Kyung Choi, and Jaiwook Park *Org. Lett.*, 9, No. 6, **2007**
7. M. J. Homann, R. Vail, B. Morgan, V. Sabesan, C. Levy, D. R. Dodds, A. Zaks, *Adv. Synth. Catal.* 2001, 343, 744-749
8. A. Goswami & T.P.Kissick *Org. Proc. Res. Dev.* **2009**, *13,* 483