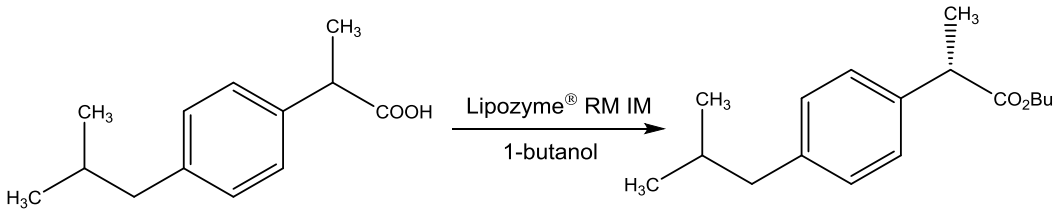
Strem Chemicals, Inc. www.strem.com

Catalog # 06-3120 Lipozyme® RM IM

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

Lipozyme® RM IM is a 1,3 specific lipase originating from *Rhizmucor miehei* which is immobilized on a resin carrier. The enzyme is a highly effective catalyst for stereoselective transesterification and ester hydrolysis.

Declared activity 275 IUN/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.



Other Lipase products offered by Strem:

06-3123 Novozym® 435
06-3155 Lipozyme® TL IM
06-3100 Novocor® AD L
06-3120 Novozym® 40086
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.

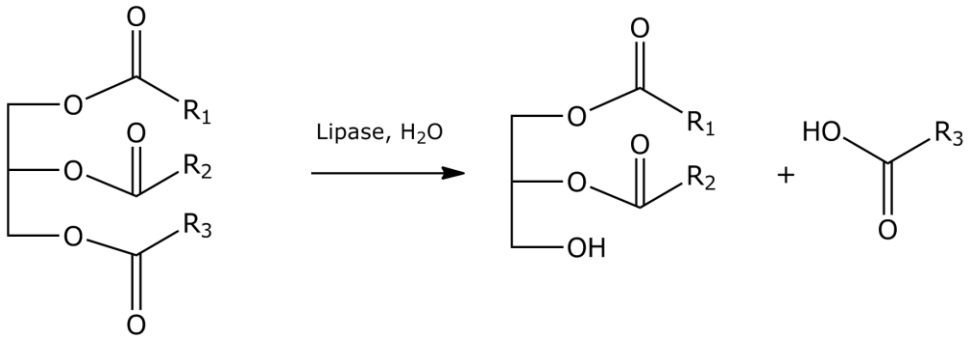


Figure 1: Regioselective hydrolysis of a triacylglycerol.

Description and optimum usage conditions

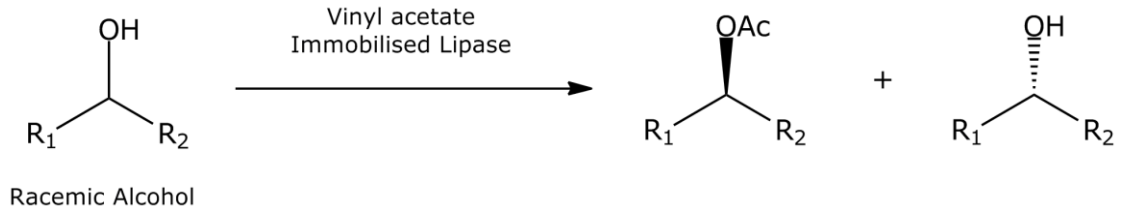
Enzyme No.	Strem Catalog Number	Product Name	Activity*	Formulation	pH optimum	Temp optimum	Substrate specificity
1.	**06-3123**	Novozym® 435	10000 PLU/g	Immobilized	pH 5-9	30-60°C	Esters and alcohols
2.	**06-3155**	Lipozyme® TL IM	250 IUN/g	Immobilized	pH 6-8	50-75°C	Esters
3.	**06-3120**	Novozym® 40086	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

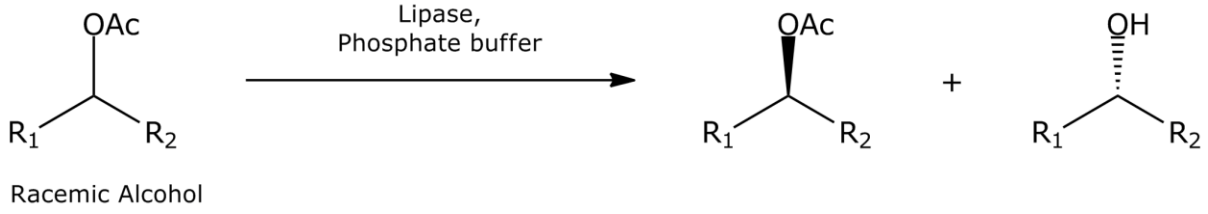
Example 1. Kinetic resolution by transesterification of racemic alcohol[1]



- 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°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.

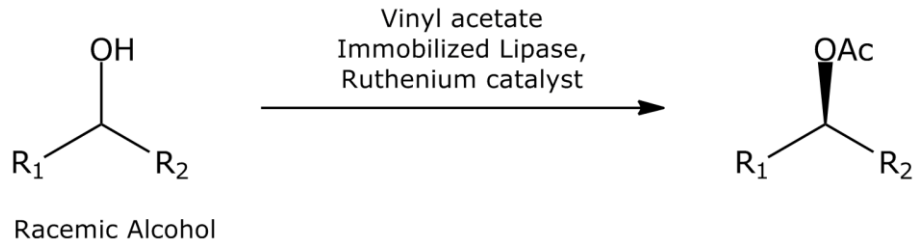
Example 2. Kinetic resolution of racemic alcohol by hydrolysis of acetoxy derivative



- 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]

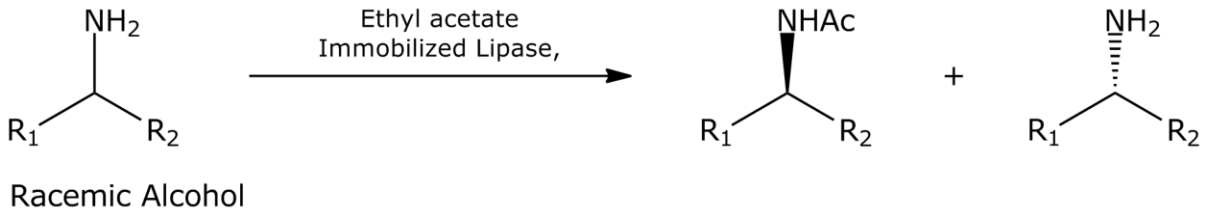


- Ruthenium catalyst* (0.05 eq with regards to substrate), Immobilized lipase **1-3** (50% w/w with regards to substrate) and Na_2CO_3 (1.0 eq. with regards to substrate) are dissolved in dry organic solvent (5 mL Toluene**) under inert atmosphere (N_2) in a closed vessel.
- Dry toluene is added and 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%)

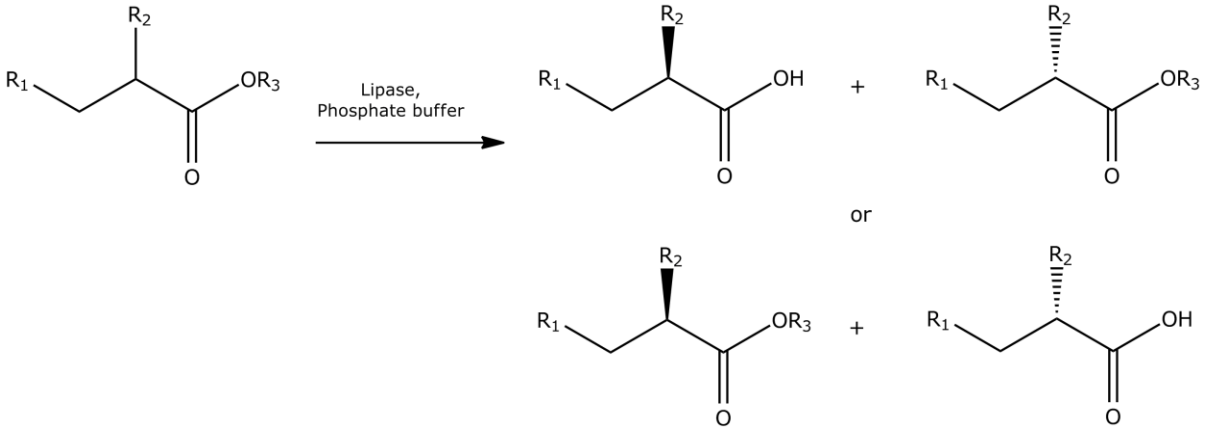
Example 4. Kinetic Resolution by transesterification of Racemic Amine[5, 6]



- 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.

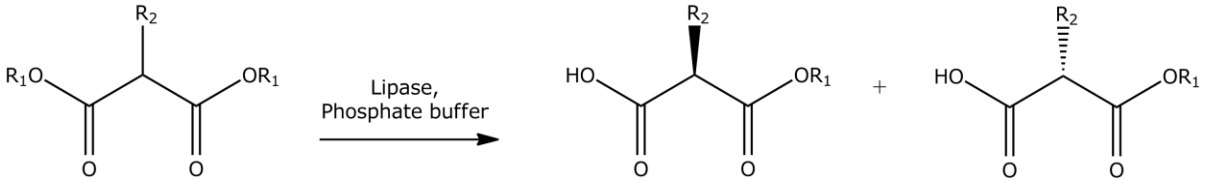
*Acyl donor solvent: α-methylbenzyl acetate, methylmethoxy acetate, ethyl acetate and methyl tert butyl ether.

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°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

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

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

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