

Catalog # 06-3118 Sustine® 131

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

Sustine® 131 is a 1,3 specific lipase originating from *Rhizmucor miehei*. It is an effective catalyst for the hydrolysis of small fatty acids on the carboxylic part.

Declared activity 20000 LU-MM/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 Sustine® 110IM
06-3155 Sustine® 120IM
06-3120 Sustine® 130IM
06-3105 Sustine® 111
06-3140 Sustine® 121
06-3135 Sustine® 140
96-0221 Lipase Screening Kit
(contains 7 lipase enzymes)

Endoprotease products offered by Strem:

06-3110 Sustine® 211
06-3112 Sustine® 210
06-3150 Sustine® 220
06-3115 Sustine® 230
06-3160 Sustine® 250
96-0225 Endoprotease Screening Kit
(contains 5 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.

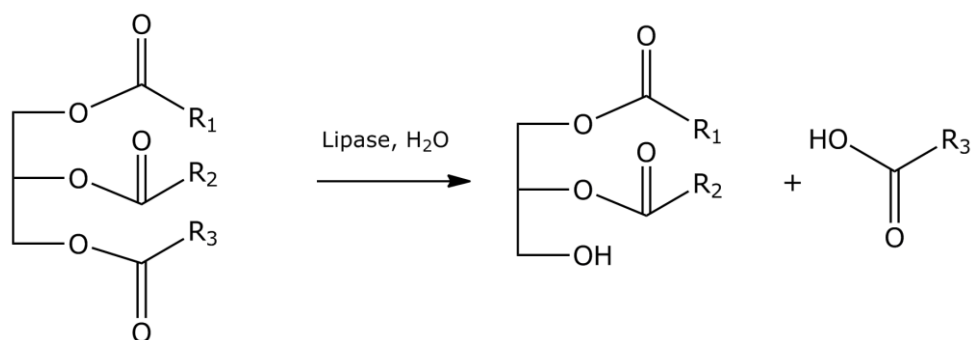


Figure 1: Regioselective hydrolysis of a triacylglycerol.

Description and optimum usage conditions

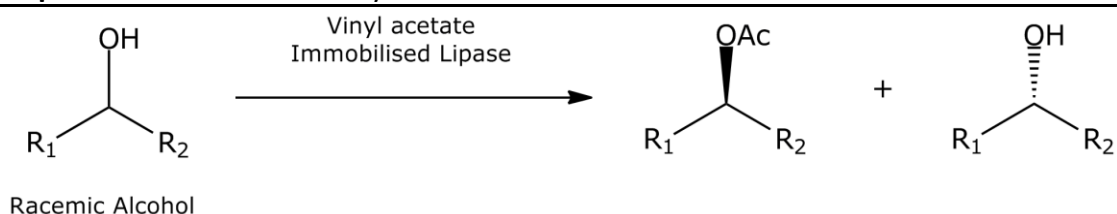
Enzyme No.	Strem Catalog Number	Product Name	Activity*	Formulation	pH optimum	Temp optimum	Substrate specificity
1.	06-3123	Sustine® 110IM	10000 PLU/g	Immobilized	pH 5-9	30-60°C	Esters and alcohols
2.	06-3155	Sustine® 120IM	250 IUN/g	Immobilized	pH 6-8	50-75°C	Esters
3.	06-3120	Sustine® 130IM	275 IUN/g	Immobilized	pH 7-10	30-50°C	Esters
4.	06-3105	Sustine® 111	5000 LU/g	Liquid	pH 5-9	30-60°C	Esters and alcohols
5.	06-3118	Sustine® 131	20000 LU/g	Liquid	pH 7-10	30-50°C	Esters
6.	06-3140	Sustine® 121	100 KLU/g	Liquid	pH 7-10	20-50°C	Esters and diesters
7.	06-3135	Sustine® 140	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 tritatable 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 alcohol¹



- 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.

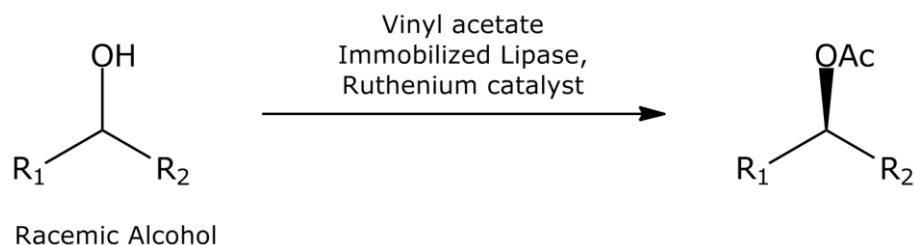
Example 2. Kinetic resolution of racemic alcohol by hydrolysis of acetoxy derivative



- 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}

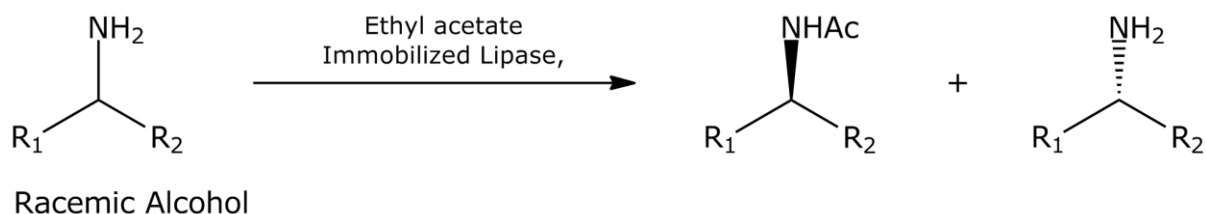


- Ruthenium catalyst* (0.05 eq with regards to substrate), Immobilized lipase **1-3** (50% w/w with regards to substrate) and Na₂CO₃ (1.0 eq. with regards to substrate) are dissolved in dry organic solvent (5 mL Toluene**) under inert atmosphere (N₂) in a closed vessel.
- Dry toluene is added and resulting 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 ($\eta^5\text{C}_5\text{Ph}_5$)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%)

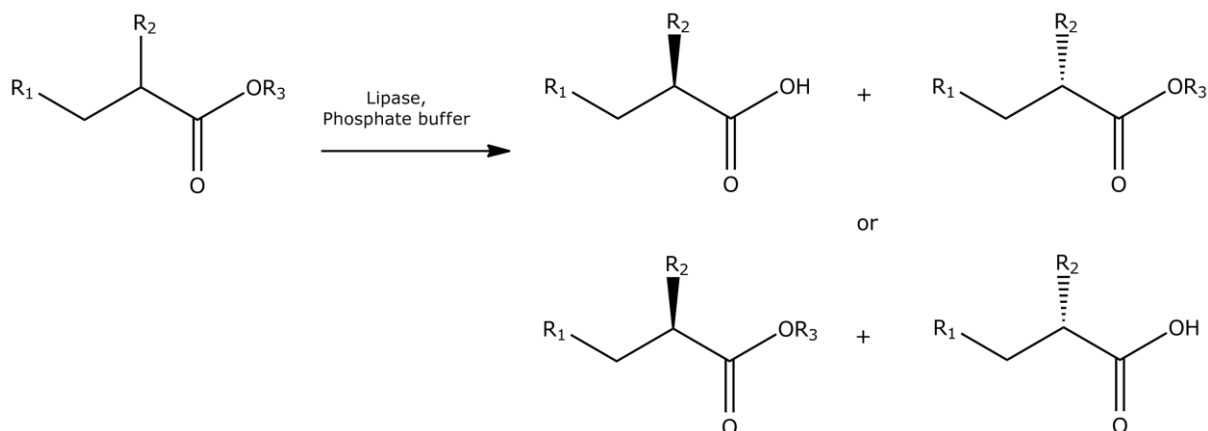
Example 4. Kinetic Resolution by transesterification of Racemic Amine^{5, 6}



- Acyl donor* ethyl acetate (5 mL) is charged to vessel under inert atmosphere (N₂)
- 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.

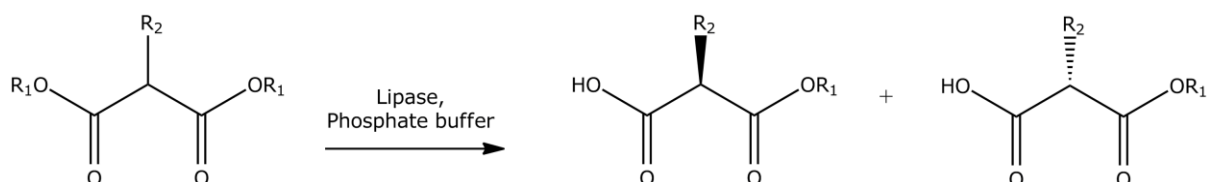
*Acyl donor solvent: α -methylbenzyl acetate, methylmethoxy acetate, ethyl acetate and methyl tert butyl ether.

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 = $((R-S)/(R+S)) \times 100$ 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

$$E = \frac{\ln \left[\frac{1 - ee_s}{1 + ee_s / ee_p} \right]}{\ln \left[\frac{1 + ee_s}{1 + ee_s / ee_p} \right]}$$

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			0.1M Sodium Phosphate Buffer at 25°C		
pH	Volume of 1M K ₂ HPO ₄ (mL)	Volume of 1M KH ₂ PO ₄ (mL)	pH	Volume of 1M Na ₂ HPO ₄ (mL)	Volume of 1M NaH ₂ PO ₄ (mL)
5.8	8.5	91.5	5.8	7.9	92.1
6.0	13.2	86.8	6.0	12.0	88.0
6.2	19.2	80.8	6.2	17.8	82.2
6.4	27.8	72.2	6.4	25.5	74.5
6.6	38.1	61.9	6.6	35.2	64.8
6.8	49.7	50.3	6.8	46.3	53.7
7.0	61.5	38.5	7.0	57.7	42.3
7.2	71.7	28.3	7.2	68.4	31.6
7.4	80.2	19.8	7.4	77.4	22.6
7.6	86.6	13.4	7.6	84.5	15.5
7.8	90.8	9.2	7.8	89.6	10.4
8.0	94.0	6.0	8.0	93.2	6.8
Dilute combined 1M stock solutions to 1 L with distilled H ₂ O.			Dilute combined 1M stock solutions to 1 L with distilled H ₂ O.		

References

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