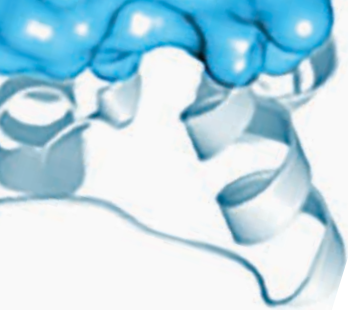


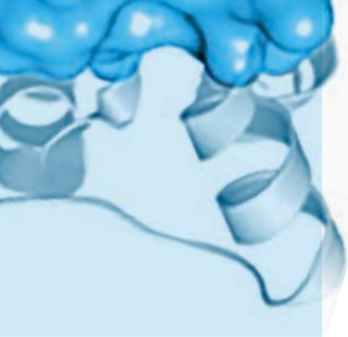
PRODUCT INFORMATION

# CalB immo KIT

Jointly developed and marketed by Purolite & c-LEcta

Purolite® Life Sciences

# Contents



# CalB immo KIT

Purolite (in collaboration with c-LEcta) has developed the CalB immo KIT, which includes a range of preparations of CalB immobilized on 6 different Purolite Lifetech™ ECR resins, each varying in:

- Activity upon immobilization
- Hydrophobicity
- Mechanical stability
- Chemical and physical properties

The CalB immo KIT is a truly unique product in the market, designed specifically to support scientists in developing new processes by facilitating rapid product screening. It includes commercially available, robust and high-performing resin products.[1]

All Lifetech ECR enzyme carriers used in the preparation of the CalB immo KIT are compliant with regulatory requirements for food and pharmaceutical industries.

Each product in the CalB immo KIT is also available on an industrial scale and can be purchased individually, if desired.

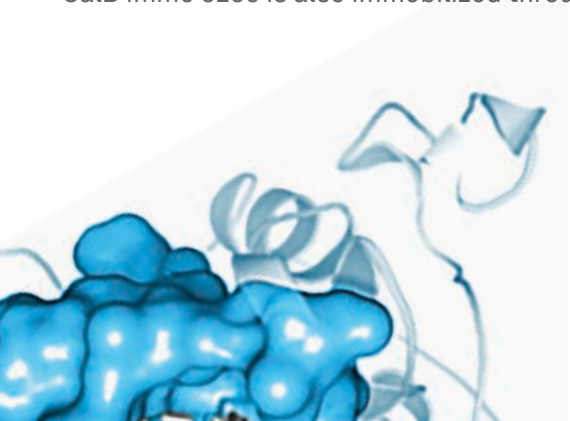
# Product Specifications

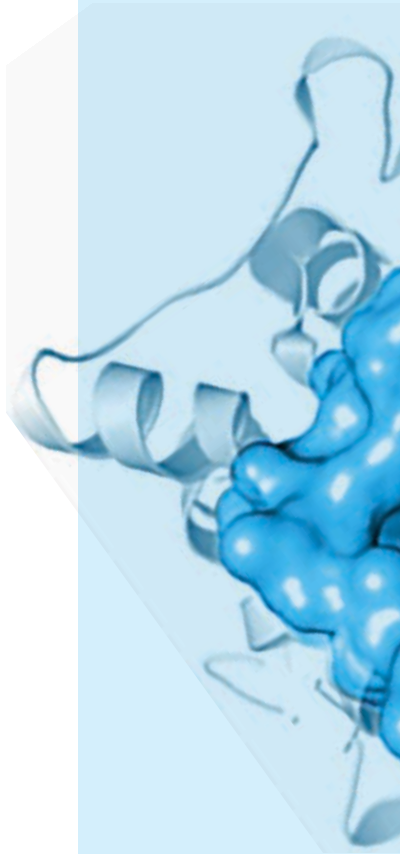
Lipase B from *Candida antarctica* (CalB) is a relatively small enzyme (approx. 33 kDa) with an optimal pH of 7 and broad pH stability (3-10).

CalB features a substantial hydrophobic surface area, making adsorption on hydrophobic carriers the ideal process for its immobilization.[2]

The CalB immo KIT includes different preparations of CalB immobilized by adsorption on hydrophobic carriers. The hydrophobic interaction responsible for the adsorption occurs via interaction with the aromatic rings of divinyl benzene or styrene (CalB immo Plus, CalB immo 5587, CalB immo 1090, CalB immo 5872), with octadecyl groups (CalB immo 8806) or with butyl groups (CalB immo 8285).

CalB immo 8285 is also immobilized through covalent immobilization onto epoxy groups.





| Table 1: Description of immobilized CalB preparations included in the CalB immo KIT | | | | | |
|---|---|---|---|---|---|
| **Product** | **Immobilization Method** | **Matrix** | **Particle Size (micron)** | **Main Features** | **Enzyme Activity (PLU/$g_{dry}$)** |
| **CalB immo Plus** | Adsorption | DVB/Methacrylate | 300-710 | High mechanical stability | >9000 |
| **CalB immo 8806** | Adsorption | Octadecyl Methacrylate | 300-710 | High enzyme activity | >10000 |
| **CalB immo 5587** | Adsorption | Macroporous Styrene | 300-710 | Cost effective | >4000 |
| **CalB immo 8285** | Adsorption & Covalent | Epoxy/Butyl Methacrylate | 250-1000 | Covalent enzyme binding | >10000 |
| **CalB immo 1090** | Adsorption | Macroporous Divinylbenzene | 300-710 | Fast product/ substrate diffusion | >8000 |
| **CalB immo 5872** | Adsorption | Polystyrene | 300-1500 | Cost effective | >3500 |

**Unit definition PLU:** One unit corresponds to the synthesis of 1 µmol per minute of propyl laurate from lauric acid and 1-propanol at 60°C. All products are supplied in dry form (< 5% moisture). Typical bulk density is 0.28 – 0.51 $g_{dry}$/ml.

# Key Features

- Mechanical stability
- No enzyme leaching in either full organic solvent or water
- Rigid polymer backbone with limited tendency to swell
- Recyclability
- High performance

# Mechanical Stability

All enzyme carriers used for the preparation of immobilized CalB are designed to have high mechanical strength so they are suitable for use in multiple cycles or packed in columns. As shown in Figure 1, the majority of carriers are much more robust than equivalent competitor resins.[4]

CalB immo 5872 and CalB immo 5587 show the greatest mechanical stability combined with competitive pricing, thus making these products effective in oil chemistry, e.g.biodiesel production.

CalB immo Plus has been designed to be both robust and highly active, making it suitable for a broad range of applications.

CalB immo 1090 and CalB immo 8806 are both more mechanically stable than competitor products, but CalB immo 8806 especially is characterized by very high activity (usually in the range of 12.000PLU/$g_{dry}$).

CalB immo 8285 shows similar mechanical stability to equivalent competitor resins, however the possibility for covalent immobilization allows its use in either aqueous media or biphasic systems.

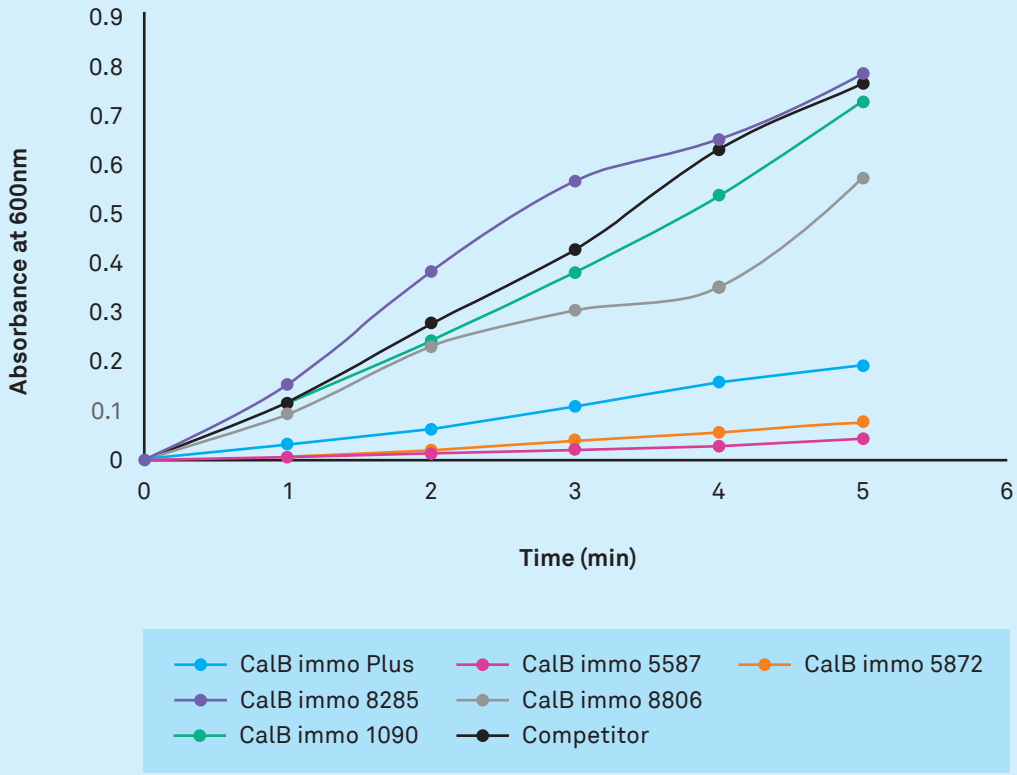



**Figure 1:** Mechanical stability profile of different preparations of CalB immo KIT and other commercial preparations. Accelerated attrition test performed in controlled conditions. The increase in absorbance at 600nm is directly related to the increase in the concentration of fines produced during the test.

# Enzyme leaching studies in water, organic solvent and solvent mixtures

Protein leaching from immobilized enzymes in the reaction media is a key challenge that can cause product contamination and regulatory issues due to the presence of unwanted proteins in the final product.

The data presented in Figure 2 demonstrates that no leaching is observed when CalB immo Plus is suspended in either full water or 2-propanol. This is due to the strong bond of the enzyme with the hydrophobic carrier.

Adsorbed preparations of immobilized CalB may not be suitable for applications in biphasic systems or aqueous/miscible organic solvent mixtures.

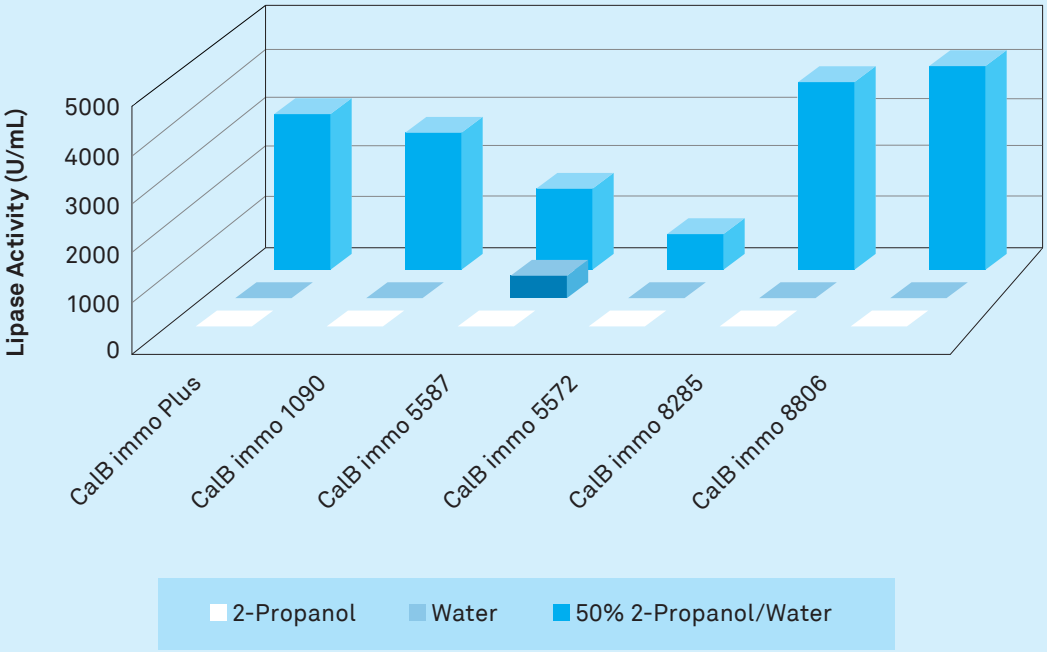



**Figure 2:** Enzyme leaching studies of CalB immo Plus in water, 2-propanol and water/2-propanol 50%. One unit corresponds to the hydrolysis of 1 µmol per minute of p-nitrophenyl butyrate to form p-nitrophenol at 20 °C.[5] Test performed using orbital shaker by mixing 1 g CalB immo Plus in 10 mL of solution for different times.

# Behaviour of immobilized enzymes in organic solvents

Swelling/shrinking of the resin is a key parameter to be considered in packed bed or expanded bed configuration. All the enzyme carriers used for the preparation of immobilized CalB are fully stable to organic solvents and the majority of chemicals.

Immobilized CalB preparations in the CalB immo KIT are supplied in dry form (moisture content <5%). When in organic solvent immobilized enzymes undergo solvation as shown in Figure 3. The rigidity of the resins minimizes changes in volume (swelling) in different solvents (hydrophilic/water miscible as acetonitrile or hydrophobic/water immiscible as hexane) and this is particularly desired when preparations are used in organic solvents and undergo washing steps. A correlation can be observed between the mechanical stability of the resins (Figure 1) and the swelling properties (Figure 3).

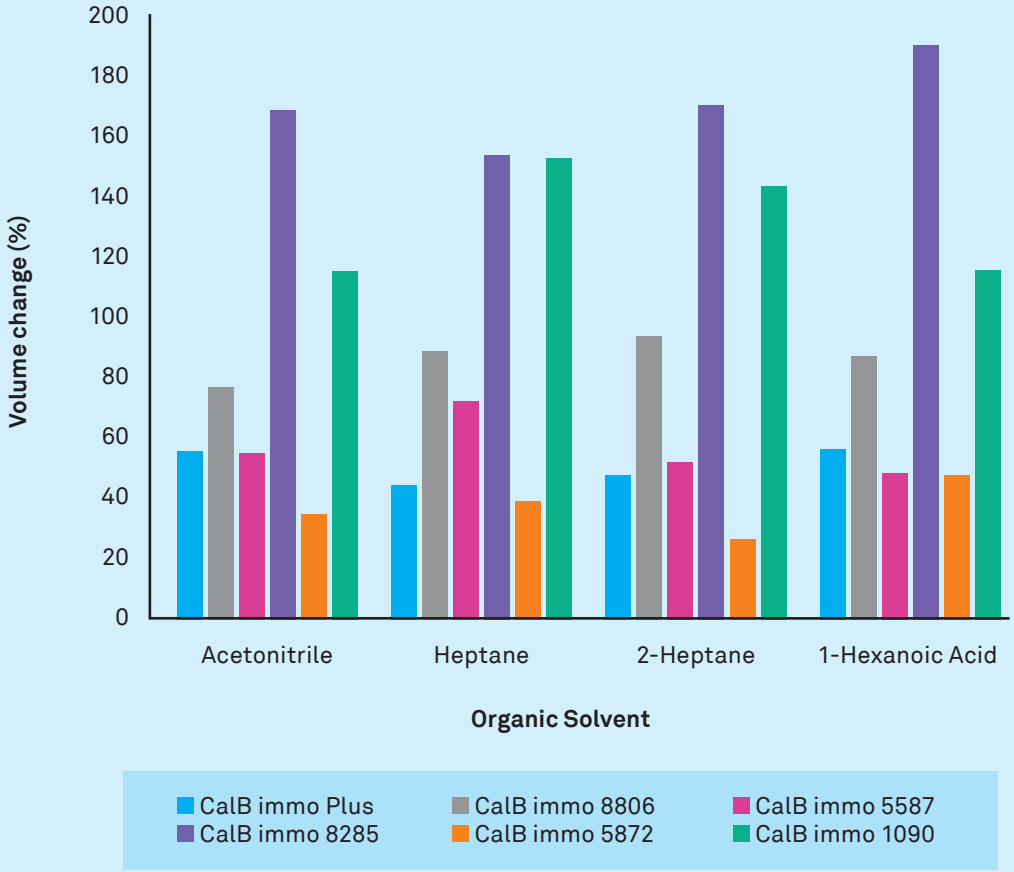



**Figure 3:** Behaviour of immobilized CalB in various organic solvents. Immobilized CalB preparations in dry form were immersed in the solvent for 24h before measuring the % increase in volume.

# Recycling studies

Immobilized enzymes have the great advantage that they can be used for multiple cycles with minimal loss of activity due to mechanical stress or enzyme deactivation.

Experiments conducted in SpinChem reactors[6] (Figure 4) have shown that CalB immo Plus can be recycled for 10 cycles without any observed loss in enzyme activity. Similar behaviour was observed for all preparations included in the CalB immo KIT.

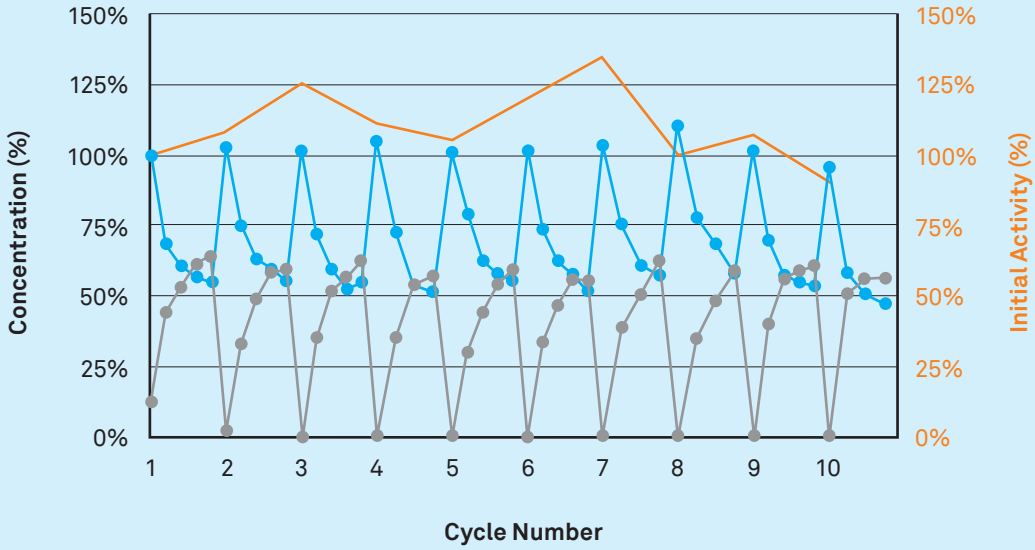



**Figure 4:** Recycling of CalB immo Plus in SpinChem reactor. Esterification reaction of 2-heptanol and acetic acid to form 2-heptyl acetate and water; cycles of 4 hours each at 30 °C.
% 2-heptanol in reaction – blue;
% 2-heptyl acetate in reaction – grey;
% initial reaction rate relative to cycle 1 – orange.

# Performance data

The immobilized enzymes included in the CalB immo KIT differ in enzyme activity as shown in Figure 5. The preparations have been tested for esterification and amidation reactions in organic solvent and compared with a main competitor product. The results shown demonstrate that the CalB immo KIT preparations perform either equal to or better than the competitor resin in both esterification and amidation.

CalB immo 5872 and CalB immo 5587 show lower performances, however they have been designed to maximize cost efficiencies, making them valuable preparations in cost-sensible applications such as oil chemistry.

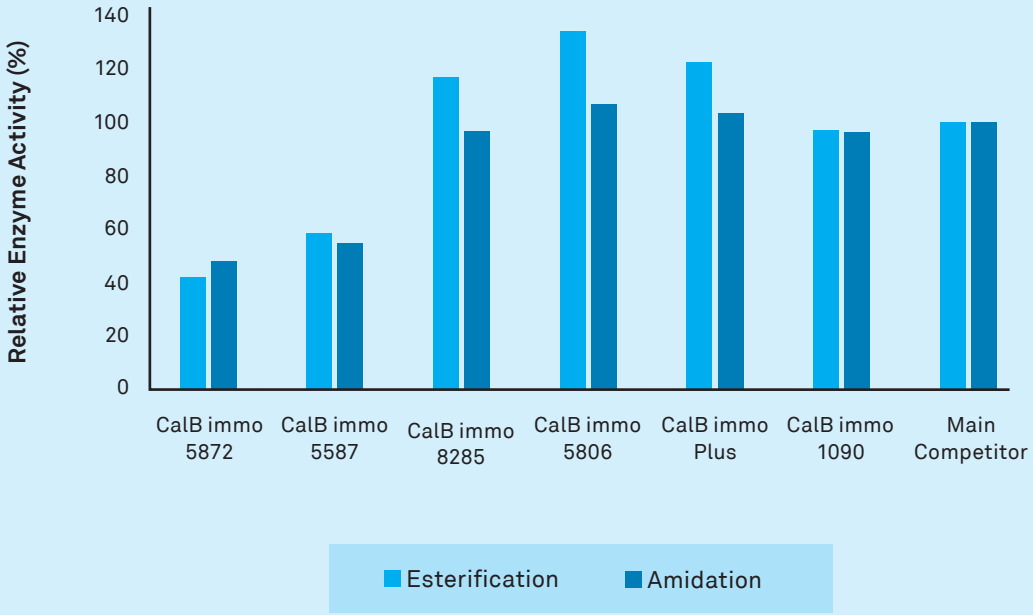



**Figure 5:** Esterification and amidation performances of immobilized CalB and a main competitor product. Data derived from proprietary reactions conducted by a third party.

# Examples of industrial applications of Immobilized CalB

Due to the broad selectivity, immobilized CalB has a wide variety of applications in industry and is used in esterification, transesterification, amidation and hydrolysis for production of chemicals, pharmaceutical molecules or intermediates, cosmetic surfactants and food ingredients.

**Examples of industrial applications (Figure 6):**

- Chemical compounds: Chiral transamidation for the synthesis of (S)-phenylethylamine[7] (Reaction 1)
- Food: transesterification of triglycerides for the manufacture of modified fatty esters[8] (Reaction 2)
- Chemical compounds: Manufacture via transesterification of derivatives of methacrylate compounds for the polymer industry[9] (Reaction 3)
- Food additives: Manufacture via transesterification of antioxidant food additive (Palmytic ascorbyl known as E number E304)[10], (Reaction 4)
- Pharmaceutical: Manufacture via hydrolysis of inhibitors of hepatitis C virus, Sofosbuvir[11] (Reaction 5)
- Cosmetic: Manufacture via esterification of esters used as surfactants[12] (Reaction 6)

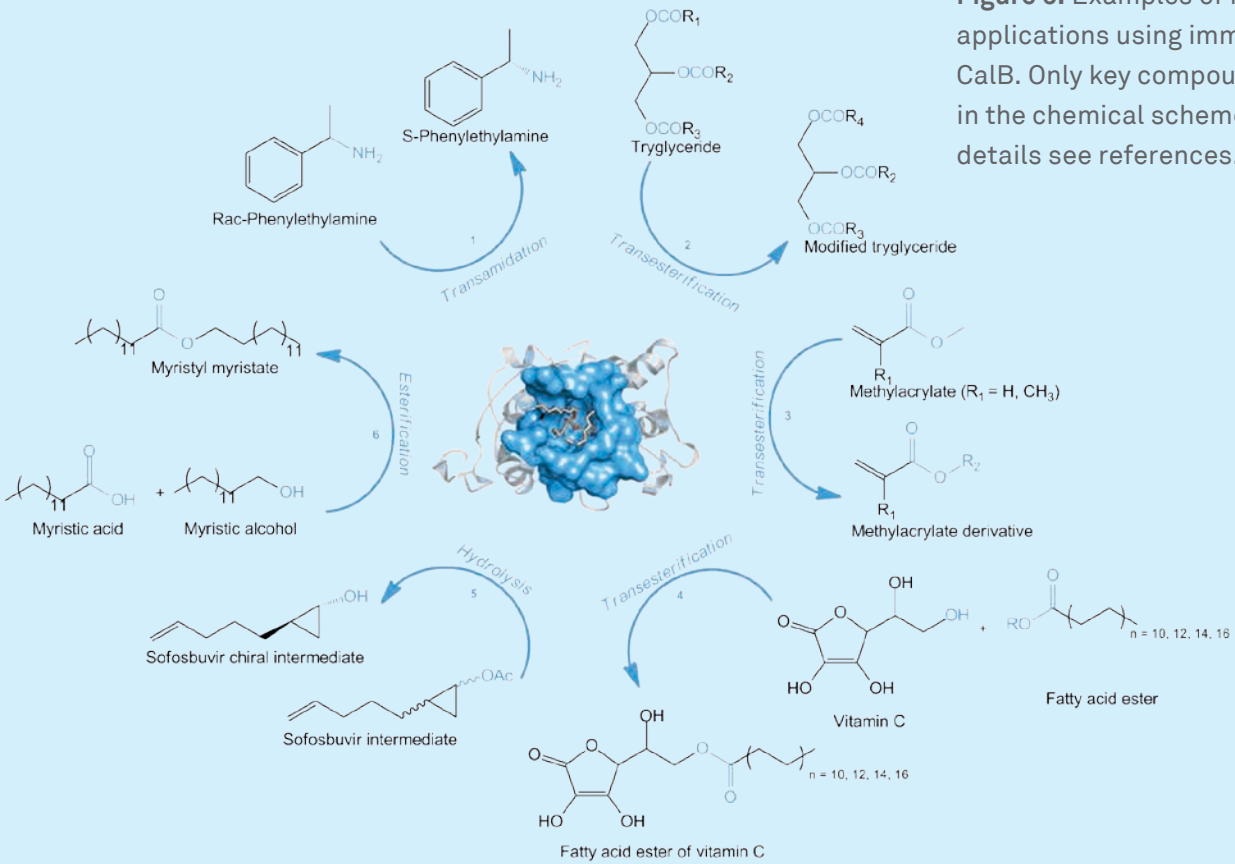



**Figure 6:** Examples of industrial applications using immobilized CalB. Only key compounds shown in the chemical schemes, for more details see references.

# Storage of immobilized CalB

CalB immo KIT preparations are sensitive to microbial contamination, so it is important that storage conditions are respected and the product is stored at temperatures 2 - 8 °C in closed packaging away from light and in dry conditions.

Microorganism contamination can also occur during use of the CalB immo KIT products, if the reaction conditions are suitable for their growth. This can be prevented by applying operating temperatures in the range 60-70 °C. Immobilized CalB preparations are suitable for operating in this temperature range.

If the preparations are to be reused, the product should be stored between cycles in either solvents or enzyme substrates that do not favour microorganism growth and do not affect the enzyme activity.

# Regulatory

The following documentation can be provided for all immobilized CalB preparations contained in the CalB immo KIT:

- Certificate for TSE/BSE and GMO free
- CalB immo Plus Food Grade manufactured under JECFA (FAO/WHO) and USP/FCC specifications
- Halal certificates

# Ordering Information

All products can be ordered through our sales offices or online, through our webshop at www.purolite.com/life-sciences.

| Ordering Information | | |
|---|---|---|
| **KIT** | **PACK SIZE** | **ORDER NO.** |
| **CalB immo KIT** | Presentation box containing 10 grams of each preparation | LS02000-KIT |
| **SINGLE ITEMS** | | |
| **CalB immo Plus** | 10 grams | LS02009-186 |
| **CalB immo Plus** | 50 grams | LS02009-192 |
| **CalB immo Plus** | 250 grams | LS02009-94 |
| **CalB immo Plus** | 1 kg | LS02009-94 |
| **CalB immo Plus** | 5 kg | LS02009-144 |
| **CalB immo Plus** | 25 kg | LS02009-700 |

| Ordering Information | | |
|---|---|---|
| SINGLE ITEMS | PACK SIZE | ORDER NO. |
| **CalB immo Plus Food Grade** | 10 grams | LS02010-186 |
| **CalB immo Plus Food Grade** | 50 grams | LS02010-192 |
| **CalB immo Plus Food Grade** | 250 grams | LS02010-94 |
| **CalB immo Plus Food Grade** | 1 kg | LS02010-94 |
| **CalB immo Plus Food Grade** | 5 kg | LS02010-343 |
| **CalB immo Plus Food Grade** | 25 kg | LS02010-700 |
| **CalB immo 8806** | 10 grams | LS02040-186 |
| **CalB immo 8806** | 50 grams | LS02040-192 |
| **CalB immo 8806** | 250 grams | LS02040-94 |
| **CalB immo 8806** | 1 kg | LS02040-94 |
| **CalB immo 8806** | 5 kg | LS02040-343 |
| **CalB immo 8806** | 25 kg | LS02040-700 |

| Ordering Information | | |
|---|---|---|
| **SINGLE ITEMS** | **PACK SIZE** | **ORDER NO.** |
| **CalB immo 8285** | 10 grams | LS02031-186 |
| **CalB immo 8285** | 50 grams | LS02031-192 |
| **CalB immo 8285** | 250 grams | LS02031-94 |
| **CalB immo 8285** | 1 kg | LS02031-94 |
| **CalB immo 8285** | 5 kg | LS02031-343 |
| **CalB immo 8285** | 25 kg | LS02031-700 |
| **CalB immo 5587** | 10 grams | LS02050-186 |
| **CalB immo 5587** | 50 grams | LS02050-192 |
| **CalB immo 5587** | 250 grams | LS02050-94 |
| **CalB immo 5587** | 1 kg | LS02050-94 |
| **CalB immo 5587** | 5 kg | LS02050-343 |
| **CalB immo 5587** | 25 kg | LS02050-700 |

| Ordering Information | | |
|---|---|---|
| SINGLE ITEMS | PACK SIZE | ORDER NO. |
| **CalB immo 1090** | 10 grams | LS02060-186 |
| **CalB immo 1090** | 50 grams | LS02070-192 |
| **CalB immo 1090** | 250 grams | LS02070-94 |
| **CalB immo 1090** | 1 kg | LS02070-94 |
| **CalB immo 1090** | 5 kg | LS02070-343 |
| **CalB immo 1090** | 25 kg | LS02070-700 |
| **Calb immo 5872** | 10 grams | LS02070-186 |
| **Calb immo 5872** | 50 grams | LS02070-192 |
| **Calb immo 5872** | 250 grams | LS02070-94 |
| **Calb immo 5872** | 1 kg | LS02070-94 |
| **Calb immo 5872** | 5 kg | LS02070-343 |
| **Calb immo 5872** | 25 kg | LS02070-700 |

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[1] Basso, A., Froment, L., Hesseler, M., Serban, S., “New highly robust divinyl benzene/acrylate polymer for immobilization of lipase CALB”, Eur. J. Lipid Sci. Technol. 2013, 115, 468-472.

[2] Basso, A., Braiuca, P., Cantone, S., Ebert, C., Linda, P., Spizzo, P., Caimi,P., Hanefeld, U., Degrassi, G., Gardossi, L., “In Silico Analysis of Enzyme Surface and Glycosylation Effect as a Tool for Efficient Covalent immobilization of CalB and PGA on Sepabeads”, Adv. Synth. Catal. 2007, 349, 877-886.

[3] Basso, A., Hesseler, M., Serban, S., “Hydrophobic microenvironment optimization for efficient immobilization of lipases on octadecyl functionalised resins”, Tetrahedron 2016, 72, 7323-7328.

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[5] Shirai, K., Jackson, R., “-“ Lipoprotein lipase-catalyzed hydrolysis of p-nitrophenyl butyrate. Interfacial activation by phospholipid vesicles”, J. Biol. Chem. 1982, 257, 1253-1258.

[6] See www.spinchem.com.

[7] See http://www.intermediates.basf.com/chemicals/chiral-intermediates/amines.

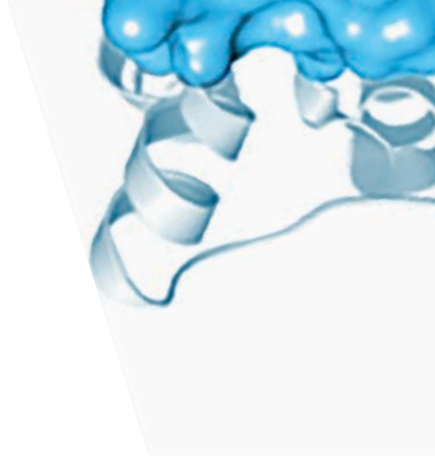
[8] Patent EP 2670854 A1, Novozymes.

[9] Patent US 2010/0273223 A1, BASF.

[10] Patent US 6479618 B1, Cognis Deutschland Gmbh (now BASF).

[11] Patent US 2014/0017198 A1, Gilead Sciences.

[12] Wiemann L. O., Nieguth R., Eckstein M., Naumann, M., Thum O., Ansorge-Schumacher M., B., “Composite Particles of Novozyme 435 and Silicone: Advancing Technical Applicability of Macroporous Enzyme Carriers”, ChemCatChem, 2009, p455



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