PRODUCT INFORMATION

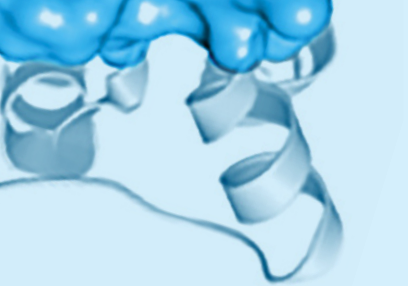
CalB immo Plus™ Immobilized Enzyme

Jointly developed and marketed by Purolite & c-LEcta

Purolite® Life Sciences

Contents

CalB immo Plus™, a high-performance and highly hydrophobic immobilized CalB preparation, has been produced through a joint development and manufacturing collaboration between c-LEcta GmbH and Purolite Corporation.



Use of Lipases for Biocatalysis

Biocatalysis is rapidly growing as a powerful industrial tool for performing synthetic organic reactions.

Lipases in general, and lipase B from *Candida antarctica* (CalB) in particular, are commonly used catalysts for production of cosmetic additives for personal care, active pharmaceutical ingredients and food ingredients due to their high regio-, chemo- and enantioselectivity. Immobilized enzymes are well established as biocatalysts in these industries. They provide sustainability, process economy, easy separation from reaction mixtures and the ability to be reused for many reaction cycles, or applied in packed-column mode.

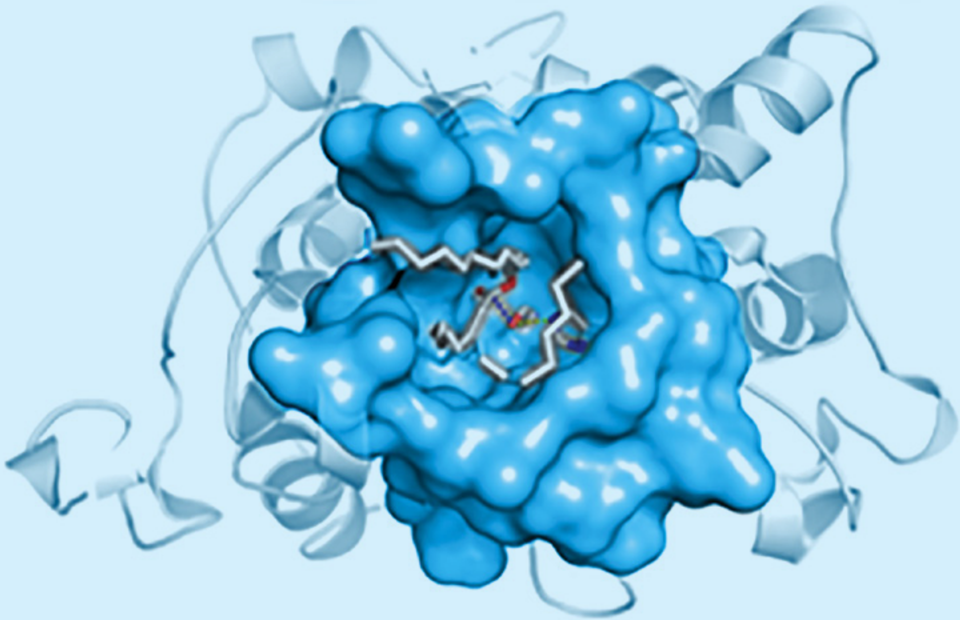


Figure 1: Schematic representation of CalB

Candida antarctica Lipase B (CalB)

Lipase B from *Candida antarctica* (Figure 1) is a relatively small enzyme (ca. 33 kDa) with an optimum pH of 7.0 and broad pH stability (3.0 – 10.0).

The native form of the enzyme is very stable, enabling its use in temperatures up to 45 – 50°C in aqueous systems—and even at higher temperatures in non-aqueous media (up to 60 – 70°C).

Moreover, CalB features substantial hydrophobic surface area, making adsorption on hydrophobic carriers the ideal process for its immobilization.

Many immobilized CalB preparations have been commercialized over the past decades. Some of them have shown good initial enzyme activity but suffer limitations in industrial applications due their mechanical weakness. For example, the strong mechanical stress encountered in typical industrial conditions causes either a decrease of enzyme activity in repeated cycles (stirred-tank reactors) or a decrease of productivity due to the formation of fines (packed bed reactors).

CalB immo Plus™, a high-performance and highly hydrophobic immobilized CalB preparation, has been produced through a joint development and manufacturing collaboration between c-LEcta GmbH and Purolite Corporation to overcome these hurdles.

CalB immo Plus enables an immobilized CalB derivative to work efficiently under strong mechanical stress at industrial scale.

CalB immo Plus™

CalB immo Plus is a recombinant lipase B from *Candida antarctica* (CalB) efficiently over-expressed in *Pichia pastoris* and immobilized on a divinylbenzene/methacrylate carrier by adsorption. The combination of an extraordinary enzyme with an excellent support makes CalB immo Plus an outstanding catalyst for synthetic challenges.

Advantages

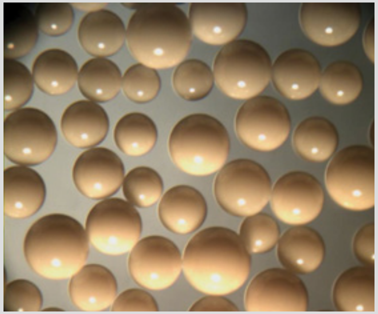
- Outstanding mechanical stability
- High enzyme activity for exceptional productivity
- Excellent chemical stability of the resin to organic solvents and temperature
- High hydrophobicity for efficient reactions and optimal mass transfer in organic solvents and water-free media
- High enzyme selectivity for the production of highly pure product compared to the use of chemical catalysts
- Ideal particle size for applications in column or batch reactors
- Controlled low <5% moisture level to prevent by-products caused by unwanted hydrolytic reactions

Table 1 – Typical Physical and Chemical Characteristics of CalB immo Plus™

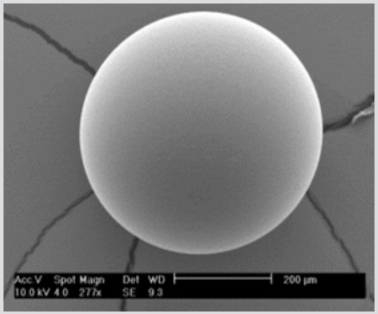
Product	CalB immo Plus
Specific activity†	> 9,000 PLU/g dry beads
Appearance	White to slightly yellow spherical beads
Polymer	DVB/methacrylate
Particle size	300 – 710 µm
Moisture	< 5%
Immobilization method	Adsorption
Typical applications	(Trans)esterifications or amidations in water-free media /organic solvents/oils
Storage	Stable for at least 12 months when stored under refrigeration (suggested 4 – 8°C)
Stability to organic solvents	Stable with nonpolar organic solvents
Temperature stability	Stable up to 60 – 80°C in nonpolar organic solvents
Regulatory	Also available in food grade quality GMO free statement TSE/BSE free statement Halal/Kosher on request

† Unit definition PLU: One unit corresponds to the synthesis of 1 µmol per minute propyl laurate from lauric acid and 1-propanol at 60°C.

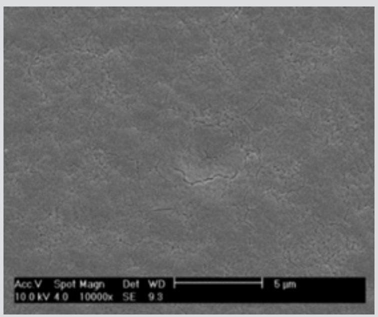
Figure 2: Microscope views of CalB immo Plus



a) Particle size distribution 300 – 710 µm



b) Close-up electron microscope view



c) Uniform surface area

To deliver CalB immo Plus™, the recombinant CalB is adsorbed onto Purolite Lifetech™ ECR1030M polymeric resin, a robust divinylbenzene (DVB)/methacrylic enzyme carrier ideally suited for the adsorptive immobilization of lipases for their further use in batch or column reactors—depending on system needs. The highly resistant support makes CalB immo Plus useful not only for column reactors, but also for use with batch processing in more rigorous stirred tank reactors.

Applications

- Ideal for a wide range of esterification, transesterification and amidation applications applied in cosmetic, personal care, pharmaceutical and food ingredients
- Suitable for use in oils, nonpolar solvents and water-free systems
- Can be used in column reactors (packed bed and fluidized bed) and batch (stirred tank) reactors

The synergy between a robust recombinant overexpression system with an outstanding polymer for enzyme adsorption makes CalB immo Plus a superior product that outperforms other commercial preparations.

Table 2 – Physical Properties of Lifetech™ ECR1030M	
Polymer	ECR1030M
Matrix	DVB/Methacrylate
Functional group	None
Immobilization	Hydrophobic adsorption
Particle size	300 – 710 μm
Surface area (m^2/g)†	> 90
Pore diameter (Å)‡	200 – 300 Å
Water content before immobilization (%)*	57 – 63 %

† Determined by B.E.T.

‡ Determined by Hg intrusion

* Determined by YDU-01L IR moisture analyzer

Enzyme Activity and Mechanical Stability

High enzyme activity and outstanding mechanical stability are the key features of CalB immo Plus™.

CalB immo Plus displays catalytic activities of > 9,000 PLU/g dry beads (based on propyl laurate synthesis), which is analogous to values reported for other commercial immobilized CalB preparations. However, and quite remarkably, to complement these outstanding enzymatic activities, CalB immo Plus is a very robust preparation with enhanced mechanical stability that can be used in both stirred batches or in column reactors (Figure 3).

The Lifetech™ ECR1030M resin support for CalB immo Plus is very strong and resilient compared to other supports used for immobilization of Lipase CalB. Because the enzyme carrier is designed to avoid formation of fine particles during repetitive cycles and usage, this product can be successfully used in packed-bed reactors.

Figure 3:
Reactor configurations suitable for the application of CalB immo Plus™

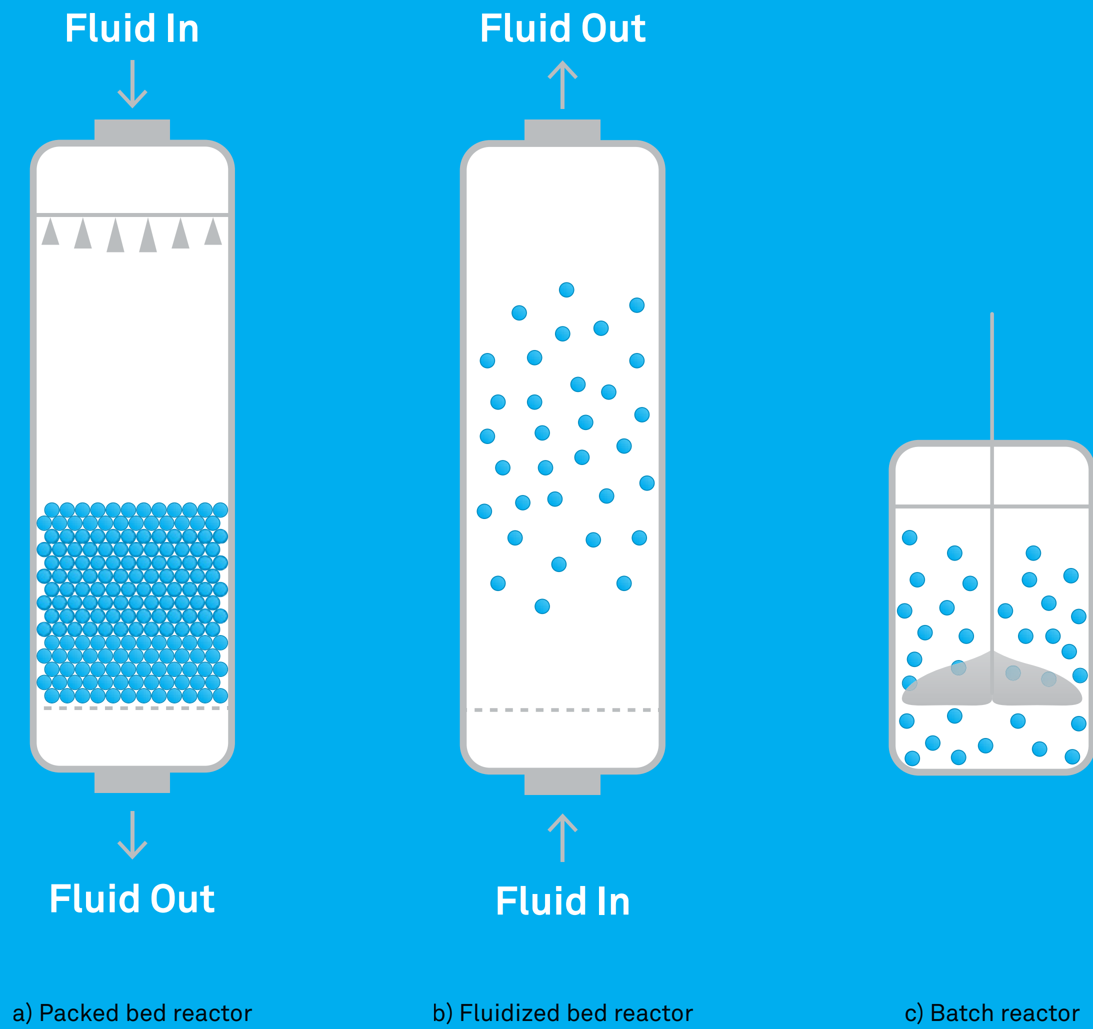
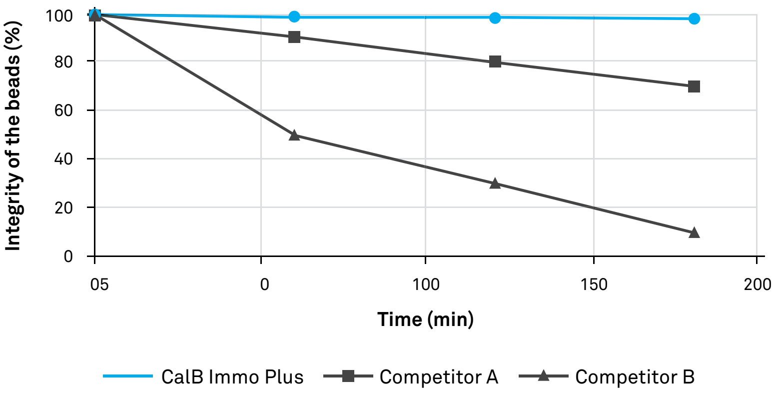
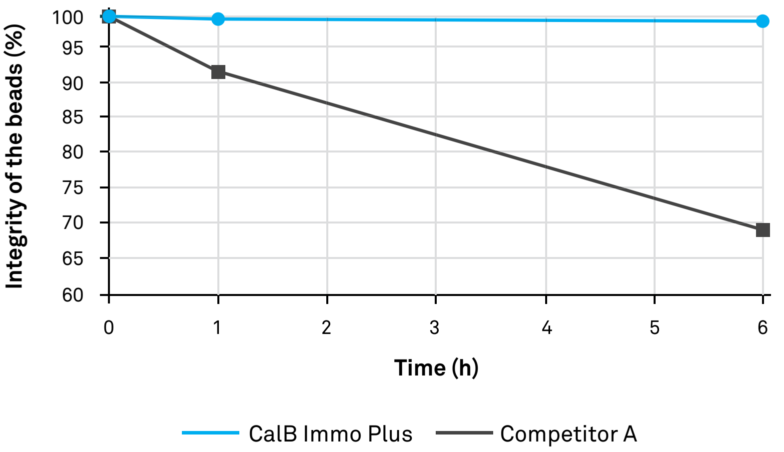


Figure 4:
Test results comparing CalB immo Plus™ to other commercial resins

a.) Batch attrition test results



b.) Ball mill test results

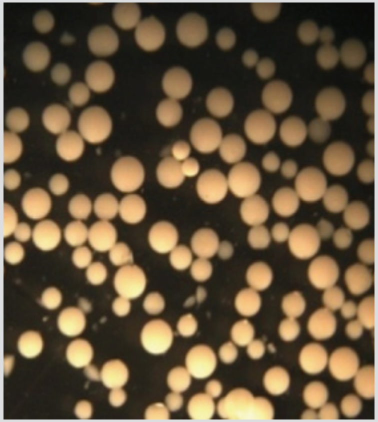


The mechanical stability of CalB immo Plus™ has been proven by comparing its performance with other commercially available immobilized CalB preparations during batch attrition and ball mill tests. Results are depicted in Figures 4 and 5 (Basso et al., 2013).

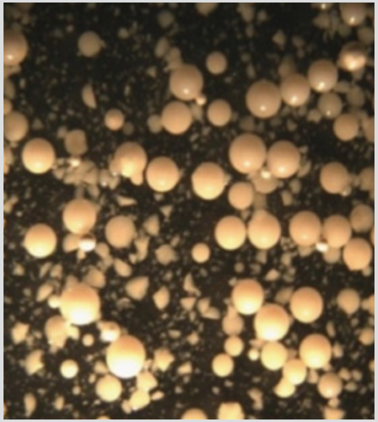
The batch attrition test was performed by mixing beads suspended in water and withdrawing samples from the solution at various time intervals followed by absorbance measurements or optical density measurements at 400 or 600 nm. An increase in absorbance was observed and is related to the formation of particulates from damaged resin beads. In the ball mill test, a hollow cylindrical steel grinder with steel balls was used to test the stability of beads. Beads were transferred into the grinder as slurry and rotated at 200 rpm. Samples were taken after 1 hr and 6 hrs to test the integrity of the beads. The change in the volume of tested resin indicates the degree of mechanical stability of the beads.

Both assays provide reliable accelerated simulations of the strong shear forces caused by the blades in (industrial) stirred reactors and the effect of pressure in packed bed reactors. CalB immo Plus shows excellent mechanical stability and integrity in batch attrition tests, remaining intact over 3 hours compared with two other commercial CalB immobilized preparations on different supports (Figure 4a and Figure 5). Additionally, ball mill tests (Figure 4b) demonstrated strength over 6 hours.

Figure 5:
a) CalB immo Plus and
b) Competitor beads after batch attrition testing



a) CalB immo Plus displays outstanding stability



b) Competitor A resin shows extensive breakage at the end of batch attrition test

Industrial Use of CalB immo Plus™

Because of poor mechanical performance of existing commercial immobilized CalB preparations, the enzyme was mostly used in academic settings, with more limited effectiveness in large-scale industrial applications. CalB is gaining more and more interest in industry due to its versatility, thermal stability, catalytic activity, broad selectivity and substrate specificity. Now, in addition to the above mentioned properties, the robust mechanical stability of CalB immo Plus makes this immobilized enzyme even more attractive to industry.

The manufacture of specialty chemicals such as cosmetic surfactants, amides, chiral amines, alcohols, polyesters, edible oils and fats will benefit from the broad specificity and stability of CalB immo Plus (Wiemann, L.O. et al., 2009). Even more, CalB immo Plus expands the application range of lipases to cover not only typical enzymatic asymmetric synthesis for developing building blocks and fine chemicals, but it also provides the possibility for use of CalB in the production of bulk chemicals.

Advantages of CalB immo Plus™ in Industrial Processes

SUBSTRATE SELECTIVITY

Broad selectivity and high specificity enable use in a wide range of applications.

LESS BY-PRODUCT

Milder reaction conditions typical for enzymes (temperature and pH) and enzyme substrate selectivity produce fewer side reactions and unwanted by-product formation, which may limit added processes and save on costs.

EASE OF SEPARATION / REUSE

Reusable hydrophobic carrier beads are efficiently recovered upon filtration, providing savings on time and cost, and producing higher yields of a purer product.

ENERGY SAVINGS

Reaction temperature range of 45 – 70 °C can decrease energy costs compared to chemical catalysts.

ENVIRONMENTAL FACTORS

Highly efficient biocatalytic processes eliminate the need for toxic, heavy metal catalysts and can help reduce environmental impact and bring industry closer to environmental goals.

NEW MARKETS

Enzymes that act as catalysts are widely regarded as green tools for chemical processes. Incorporating “green catalysts” can help demonstrate a commitment to environmental management and build customer loyalty and trust.

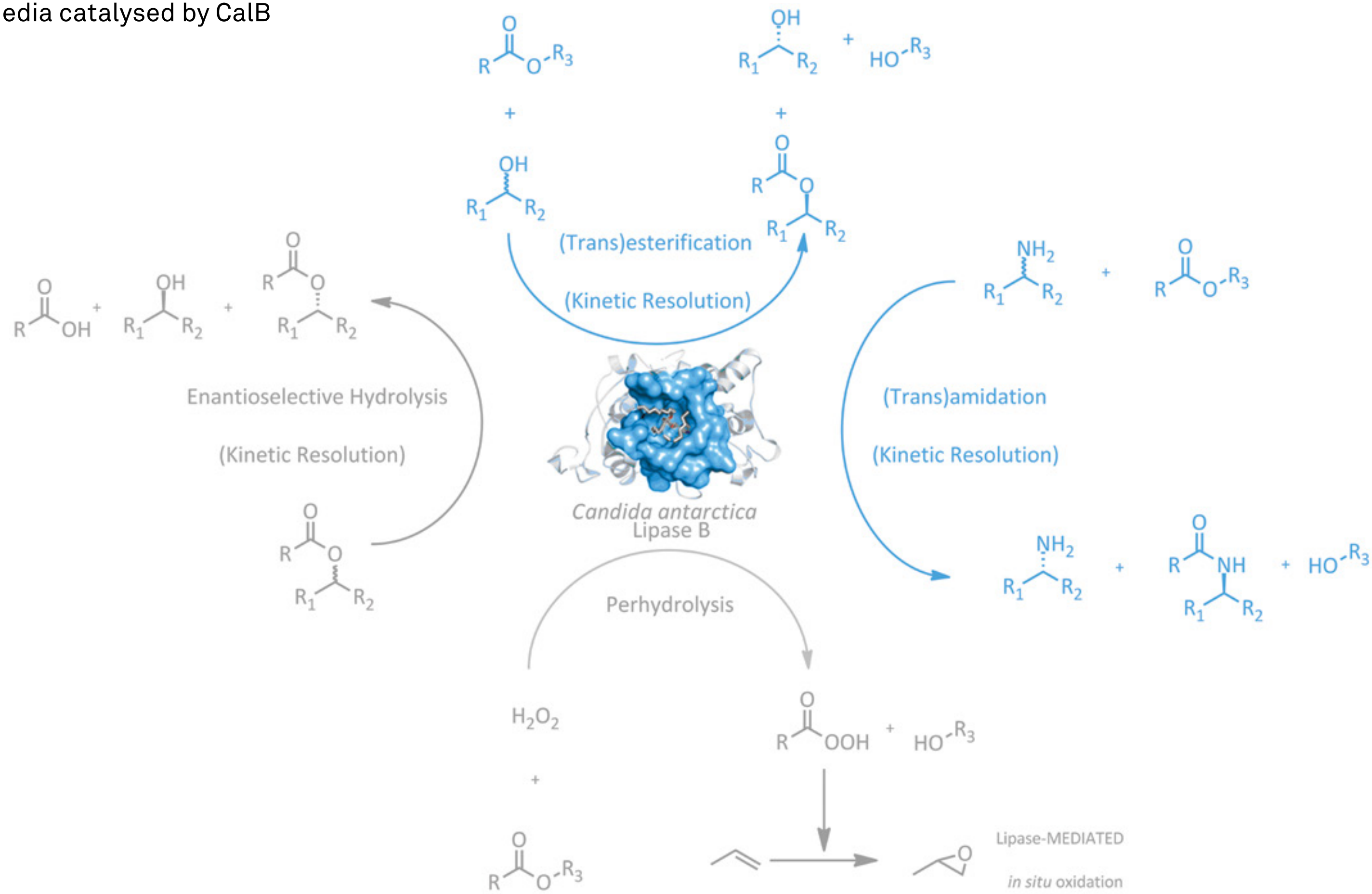
FINE CHEMICALS AND PHARMACEUTICALS:

From Alcohols and Carboxylic Acids to Amines in Enantioselective Synthesis

Enzymes are polymers composed of chiral amino acids. The active site of the enzyme—where catalysis is actually taking place—is a multi-chiral micro-environment. This provides enzymes with the capacity to transfer chirality to molecules and easily produce optically active building blocks for fine chemicals and pharmaceuticals (Jaeger et al., 1999).

Based on these features, it is no surprise that the first application considered for lipases was biocatalytic asymmetric synthesis. Their inherent robustness—allowing the use of non-aqueous solvents or synthetic applications—makes them appealing catalysts for chemists. Figure 6 provides an overview of possible uses of CalB for asymmetric synthesis.

Figure 6:
Overview of possible reactions and media catalysed by CalB



Different options are possible with CalB (Figure 6). If operating under aqueous conditions (e.g. buffer with organic water-miscible co-solvents to solubilize organic molecules), then the enantioselective hydrolysis of esters can be set (Figure 6, grey). This will afford optically active alcohols, as the enzyme will only accept one of the enantiomers, and only one type of ester will be hydrolyzed. The remnant ester can be racemized later on, to be recycled in the enzymatic process. Likewise, an analogous approach can be used for the hydrolysis of prochiral esters, leading to complete conversions with the enzymes (Note that although CalB immo Plus™ is not designed to be used for hydrolytic reactions; Purolite and c-LEcta can provide an immobilized CalB preparation suitable for hydrolysis).

Based on the enzymatic promiscuity, upon addition of diluted hydrogen peroxide in non-aqueous media together with acyl donors (e.g. esters), peracids can be formed *in situ* with CalB. These peracids can subsequently be used in the oxidation of alkenes to create epoxides or for the mild oxidation of aldehydes (to acids) and ketones (for Baeyer-Villiger reactions), to cite some examples (Figure 6, light grey).

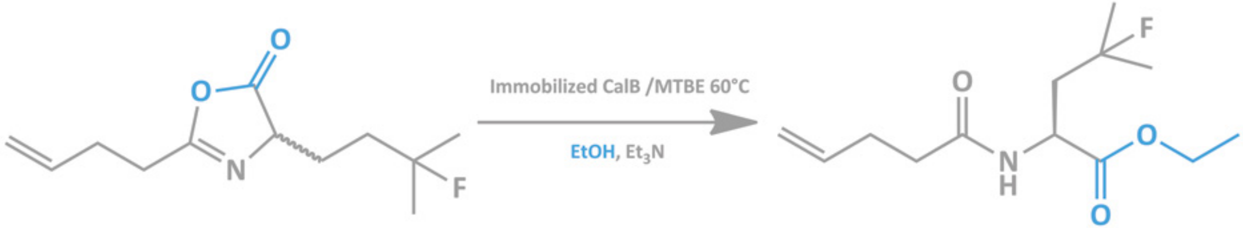
Apart from aqueous conditions, CalB can also be used in a broad range of organic nonpolar solvents. This is the optimal application for CalB immo Plus. As water is not present, another nucleophile must be added, namely alcohols, amines, etc. In this way, it is possible to run enantioselective synthetic reactions such as transesterifications, esterifications and amidations (Figure 6, blue). Here, inexpensive acyl donors such as ethyl acetate or vinyl esters are used, and the enzyme performs the (trans)esterification only based on one of the enantiomers. This kinetic resolution can be converted in a dynamic kinetic resolution (DKR) if another catalyst is added to racemize the remnant substrate.

For synthetic purposes, CalB immo Plus accepts a broad range of substrates. With regard to alcohols, an ample number of primary and secondary alcohols are actual substrates for CalB immo Plus.

CalB immo Plus is also able to accept a broad range of carboxylic acids like unsaturated, short-chain and fatty acids.

Apart from the previously-described building blocks for fine chemicals—namely optically active alcohols, amines, etc., that need to be further chemically derivatized—CalB immo Plus can also be used for the synthesis of pharmaceutical compounds. For example, the manufacturing process for a key fluoroleucine intermediate for pharmaceuticals—recently developed by Merck—is using CalB for the chirality step during production. Here, the immobilized CalB is used and recycled in an organic solvent (MTBE) at 60°C, via transesterification of a lactone with ethanol as a nucleophile (Figure 7) (Truppo et al. 2008).

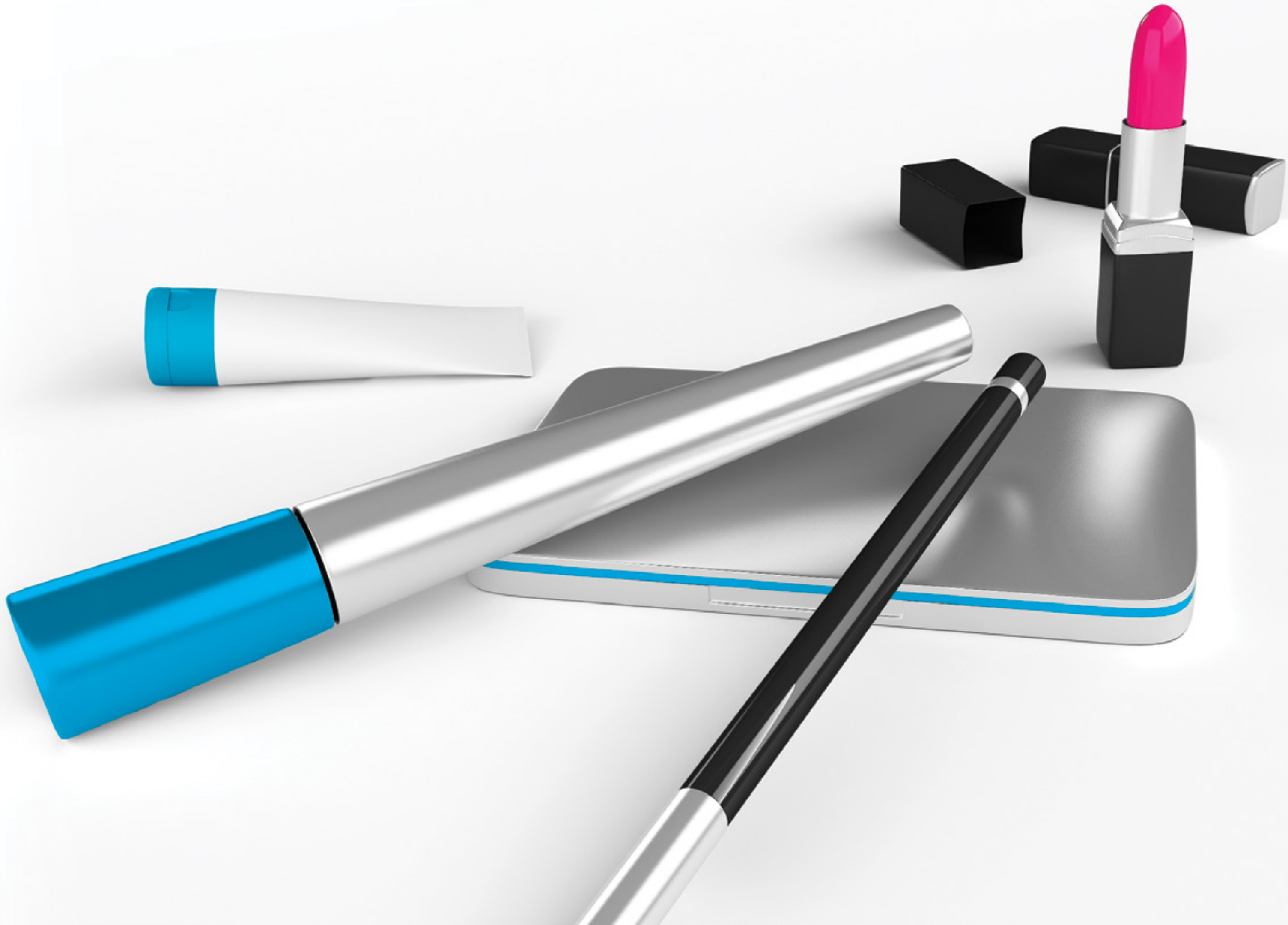
Figure 7:
Example of immobilized CalB applied in industrial chiral production of pharmaceutical building blocks



Triglyceride Synthesis for Food Industries

In nature, lipases catalyse the (selective) hydrolysis of triglycerides and fats into fatty acids and glycerol. However, lipases in absence of water can catalyse the opposite reaction of synthesis of triglycerides and fats. In industrial production of fats and oils, there is increasing demand for modified fats and oils with new nutritional, melting or organoleptic properties. These modifications can be done using chemo-catalysts. However, unwanted by-product formation, low selectivity on the desired reaction and usually off-spec unmarketable compounds can result. The selectivity of lipases and their effectiveness under mild conditions makes them practical and efficient for tackling these challenges. Different lipases are currently being used in industrial food production and fat modification processes.

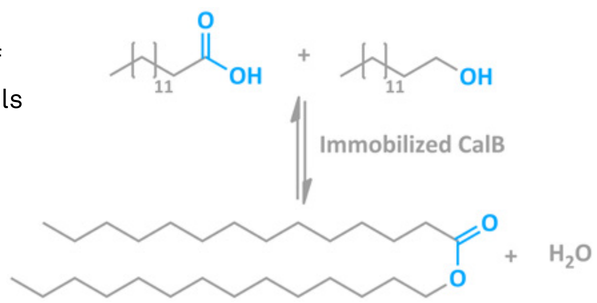
Due to the benefits of 1,3-diacylglycerol (1,3-DAG) oils for obesity prevention, more attention has been given to enzyme-mediated development of 1,3-DAG from triglycerides that will not form by-products. Lipase CalB is used to create novel value-added products by using transesterification of different vegetable oils (like soybean oil) with different alcohols, e.g. ethyl ferulate. Another important example for the usage of CalB in food industries is the production of triglycerides enriched in polyunsaturated fatty acids (PUFA), which are marketed as functional food. Additionally, the enzymatic hydrolysis of fats and oils becomes economically viable due to the reusability of immobilized enzymes.



Bulk Chemical and Surfactant Manufacturing for the Cosmetic Industry

Immobilized CalB is used in the industrial production of emollient esters for cosmetic purposes, such as myristyl myristate or cetyl ricinoleate. Because of the high regioselectivity of Calb immo Plus™ under mild reaction conditions, it is effectively used in other chemical segments such as surfactants for cosmetics (Figure 8). Additionally, CalB immo Plus prevents the formation of by-products, and in some cases unwanted color formation.

Figure 8:
Example of esterification of fatty acids and fatty alcohols catalysed by immobilized CalB to produce wax ester (Wiemann et al. 2009)



Quality Management

CalB immo Plus™ is the most highly optimized solution for biocatalytic processes, conforming to strict quality control protocols for both Purolite and c-LEcta. All R&D sites, production plants and main international sales offices of Purolite and c-LEcta are ISO 9001:2015 accredited. In addition, Purolite has cGMP and FDA inspection at its Romanian manufacturing site for the production of pharmaceutical grade resins.

The superior quality and consistency of CalB immo Plus is the result of a robust manufacturing processes and attention to detail, as well as strict controls over raw materials, intermediates and final products.

Regulatory Support

CalB immo Plus™ is manufactured to the following regulatory guidelines:

- Council of Europe Resolution ResAP (2004)3: ECR1030 conforms to this standard regarding ion exchange and adsorbents resins used in the processing of foodstuffs
- Animal-free statement (TSE/BSE-free certificate): No animal derived materials or reagents are used in the entire production process of CalB immo Plus
- GMO free statement: CalB immo Plus does not contain any genetically modified organisms
- JECFA (FAO/WHO) and USP/FCC specifications: A food-grade CalB immo Plus is available
- Kosher and Halal product certificates: Certificates can be provided upon specific request

Purolite®

About Purolite

Purolite was founded in 1981 and is a leading manufacturer of ion exchange, catalyst, adsorbent and specialty resins. Headquartered in Bala Cynwyd, PA, the company has ISO-9001 certified manufacturing facilities in the USA, China and Romania, and operates dedicated R&D centers in the USA, China, Romania, Russia and the UK. Purolite, the only company focused exclusively on resin technology, has 40 sales offices in more than 30 countries with the most skilled technical sales force in the industry. Purolite Life Sciences includes the widest portfolio of resins for enzyme immobilization and biochromatography.

Please visit www.purolite.com and www.purolitelifesciences.com.

About c-LEcta

c-LEcta is an industrial biotechnology company that applies best-in-class biotechnologies to efficiently provide customized enzymes and microbial strains for industrial applications. The company covers a broad range of the value chain from discovery and engineering to commercial production of customized enzymes and strains. Scientific excellence is combined with in-depth commercial and regulatory know-how to bring innovative and competitive bioprocessed products into scale.

Please visit www.c-LEcta.com for more information.

CalB immo Plus is shipped as dry product with moisture <5%.

Table 3 – CalB immo Plus™ Information

Product Name	Available Quantities	Container Description
CalB immo Plus	10 g	50 g tub
	50 g	250 g tub
	250 g	1 kg tub
	1 kg (4 tubs)	1 kg tub
	1 – 9 kg	5 kg white tub
	> 10 kg	50 kg blue keg
CalB immo Plus Food Grade	10 g	50 g tub
	50 g	250 g tub
	250 g	1 kg tub
	1 kg (4 tubs)	1 kg tub
	1 – 9 kg	5 kg white tub
	> 10 kg	50 kg blue keg

References

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for tomorrow´s industry

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