PRODUCT INFORMATION

Purolite Lifetech™ ECR Enzyme Immobilization Resins

Immobilized enzymes are robust catalytic agents that enable easy separation of the enzyme from the product as well as reuse of the enzyme. They are also powerful tools for optimizing process operations. This document reviews the Purolite Lifetech™ portfolio of resins for covalent, adsorption and ionic immobilization for pharmaceutical, biopharmaceutical and chemical applications.

Purolite® Life Sciences

Inside this Product Information Guide you will find an overview of Purolite® Lifetech™ ECR Enzyme Immobilization Resins that enable easy separation of the enzyme from the product as well as reuse of the enzyme. For more detailed information on any product or to find a product for an application not mentioned, please go to www.purolitelifesciences.com or contact the Purolite office closest to you, listed on the back cover.

INTRODUCTION

Founded in 1981, Purolite is a leading manufacturer of ion exchange, catalyst, adsorbent and specialty resins. With global headquarters in the United States, Purolite is the only company that focuses 100% of its resources on the development and production of resin technology.

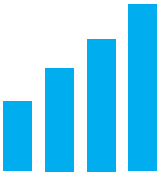
Responding to the needs of our customers, Purolite has built the largest technical sales force in the industry, the widest variety of products and five strategically located Research and Development groups. Our ISO 9001 certified manufacturing facilities in the U.S.A, UK, Romania and China combined with more than 40 sales offices in 30 countries ensure worldwide coverage.

PREMIER PRODUCTS

The quality and consistency of our products is fundamental to our performance. Throughout all Purolite plants, production is carefully controlled to ensure that our products meet the most stringent criteria, regardless of where they are produced.

RELIABLE SERVICE

We are technical experts and problem solvers. Reliable and well trained, we understand the urgency required to keep businesses operating smoothly. Purolite employs the largest technical sales organization in the industry.



INNOVATIVE SOLUTIONS

Our continued investment in research & development means we are always perfecting and discovering innovative uses for ion exchange resins and adsorbents. We strive to make the impossible possible.

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Immobilized enzymes

Immobilized enzymes are powerful tools to optimize processes in both operative and economic terms.

In addition to a more convenient handling of enzyme preparations, the two main targeted benefits of immobilized enzymes are (1) easy separation of the enzyme from the product, and (2) reuse of the enzyme. Immobilized enzymes allow easy separation of the catalyst after the reaction, e.g. filtration, thereby reducing the costs of downstream processing. Moreover, the easy separation of the enzyme from the product simplifies enzyme applications and enables a reliable and efficient reaction technology. On the other hand, the reuse of immobilized enzymes provides cost advantages that are often an essential prerequisite for establishing an enzyme-catalyzed process in the first place.

The applications using immobilized enzymes offer a high degree of flexibility, being suitable for continuous processes using fixed or expanded-bed reactors or in batch processes using stirred-tank configurations.

The immobilization of the enzyme on a rigid structure increases the biocatalyst stability, especially in organic solvents, by preventing the protein from unfolding to a certain degree. Lifetech ECR resins include methacrylic or styrene resins, with different degrees of hydrophobicity and porosity specially designed for immobilization of hydrophobic enzymes such as lipases.

There are critical parameters in the preparation of immobilized biocatalysts that have a high impact on the efficacy of the system and are tightly controlled. These include immobilization yield, mass transfer limitations and operational stability. Operative binding forces vary between multiple weak adsorptive interactions and specific attachments through strong covalent binding. The most appropriate method to use is usually determined by the application parameters.

Nowadays, immobilized enzymes produce important pharmaceuticals and food additives in large ton scale. Some of these industrial processes are listed in Table 1.

Table 1 – Examples of immobilized enzymes in industrial applications

ENZYME	SUBSTRATE	PRODUCT	AMOUNT (tons/yr)	APPLICATION
Glucose isomerase	Glucose	HFCS	8,000,000	Food
Nitrile hydratase	Acrylonitrile	Acrylamide	30,000	Chemicals & wastewater treatment
Penicillin amidase	Penicillin G	6-APA	6,000	Pharmaceutical
Aspartase	Fumaric acid	L-Aspartic acid	1,200	Chemicals
Fumarase	Fumaric acid	L-Malic acid	360	Chemicals
Aminoacylase	Acyl-D-L-amino acid	L-Amino acid	300	Food
Lipase	Rac-1-phenylethylamine	S-1-Phenylethylamine	200	Chemicals & food
Aspartase ß-decarboxylase	Aspartic acid	L-Alanine	120	Chemicals
Cephalosporin amidase	Glutaryl-7-ACA	7-ACA	Unknown	Pharmaceutical
Lactase (galactosidase)	Lactose	Lactose free milk	Unknown	Dairy

Legend: HFCS (High Fructose Corn Sirup); 6-APA (6-aminopenicillanic acid); 7-ADCA (7-Aminodesacetoxycephalosporanic acid).

Parameters affecting the performance of immobilized enzymes

The properties of immobilized enzyme preparations are determined by the properties of both the enzyme and the carrier material. The specific interaction between the two provides an immobilized enzyme with distinct chemical, biochemical, mechanical and kinetic properties (Figure 1).

In terms of manufacturing costs, the efficiency of immobilization is mostly determined by the immobilization method and the amount of soluble enzyme used. Under process conditions, the resulting activity may be further reduced by mass transfer effects. More precisely, the efficiency of immobilization depends not only on losses caused by the binding procedure but also from diminished availability of enzyme molecules within pores or from slowly diffusing substrate molecules. Such limitations, summarized as mass transfer effects, lead to lowered efficiency.

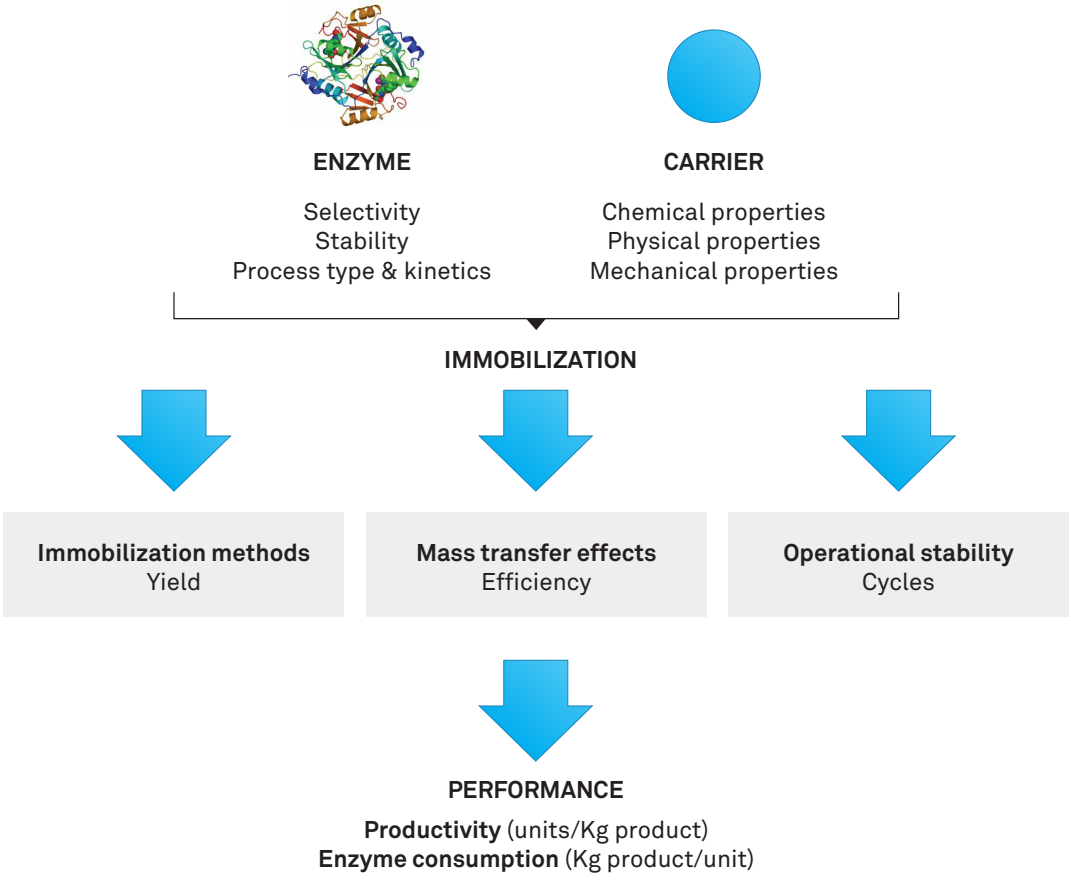
On the other hand, improved stability under working conditions may compensate for such drawbacks, resulting in an overall benefit. Altogether, these interactions are a measure of productivity or of enzyme consumption, for example, expressed as enzyme units per kg of product. If one replaces “enzyme units” by “enzyme costs” one obtains the biocatalyst impact cost per kg of product.

Major properties of carriers for enzyme immobilization

The characteristics of the carrier greatly influence the performance of an immobilized enzyme. The following properties should be well selected and balanced for a specific biotransformation (see Figure 1).

Functional groups: The type of activation, presence, distribution and density of functional groups determines the efficiency of immobilization, the stability and operational stability of the carrier-fixed enzyme. Lifetech ECR resins are offered in a variety of functional groups and customizations are available.

Figure 1 – Parameters affecting the performance of immobilized enzymes



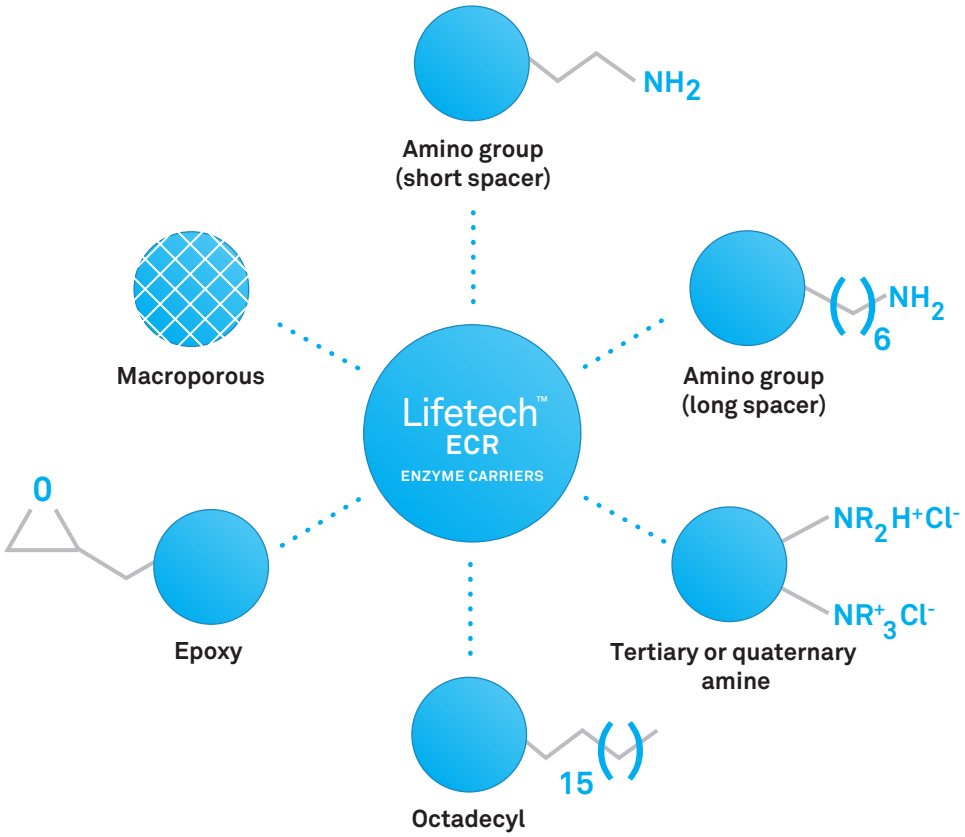
Porosity and Surface area: In most cases, large surface area (> 100 m^2/g) and high porosity are desirable so that enzyme and substrate can be easily penetrated. Pore size of > 300 Å is usually adequate to make the internal surface accessible for immobilization of most enzymes. Lifetech ECR resins are available with different degrees of porosity and surface area, usually higher than 400Å and > 40 m^2/g respectively.

Hydrophilicity/hydrophobicity: The matrix influences the type and strength of non-covalent protein-matrix interaction. In addition, it can influence the adsorption, distribution and availability of the substrate and product. Lifetech ECR resins are made of styrene, methacrylate or styrene/methacrylate thus offering a full range of hydrophilicity in order to cover every kind of application.

Insolubility: This is essential, not only for prevention of enzyme loss, but also to prevent contamination of the product by dissolved matrix and enzyme. Lifetech ECR resins are rigid and spherical beads and can be used for applications in batch or column reactors.

Mechanical stability/rigidity: These properties are dependent on the type of reactor. If used in a stirred tank reactor, the support should be stable against sheer forces to minimize abrasion. Production of fines (particles below 50 - 100 µm) can lead to the obstruction of sieve plates and filters. Lifetech ECR resins are designed to be mechanically stable, allowing their use in repeated cycles.

Figure 2 – Available chemistries of Lifetech ECR resins for enzyme immobilization



Shape and size of support: Particle size and shape influence the filtration time from stirred tank reactors in repeated batch mode. It also affects back pressure and flow rate performance in column reactors. For batch reactors, where quantities of immobilized enzymes are from few kilo scale up to hundreds of kilos, spherical particles in the range of 150 - 300 µm are preferred as they give highest activity and optimal filtration time. For column reactors where quantities of immobilized enzymes range from hundreds of kilos to tons of kilos, a size of 300 - 710 µm is preferred to avoid high back pressures. For food applications where viscosity is a key issue, including the sugar industry, an industrial grade and ionic immobilization is recommended (300 - 1200 µm). Full particle size range is also available as 150 - 710 micron.

Resistance to microbial attack: During long-term usage the support has to be stable against microbial contamination. Lifetech ECR resins are inert materials and can be stored for long periods without any loss in performance.[1]

Regeneration: This property is of interest especially in the case of expensive carrier materials or in specific applications. Lifetech ECR resins for adsorption can be regenerated and reused for further enzyme immobilization. Lifetech ECR resins for ionic immobilization can also be regenerated using standard procedures.

Safety and regulations: Lifetech ECR enzyme carriers comply with the Council of Europe Resolution ResAP (2004) 3 on ion exchange and adsorbents resins used in the processing of food materials. Kosher and Halal certifications are also available.

Epoxy-activated resins

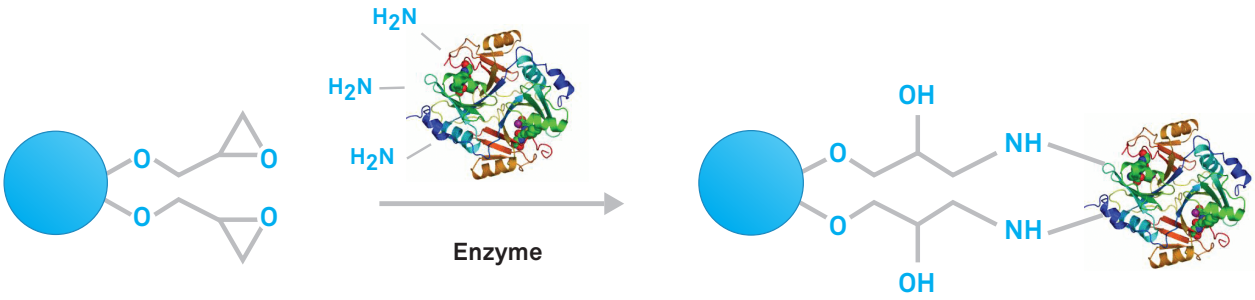
Epoxy-activated resins are almost ideal matrices to perform easy immobilization of enzymes since they allow multipoint covalent binding between the enzyme and resin.

Purolite has performed intensive R&D work to improve the resin backbone and achieve very robust resins functionalized by epoxy or by amino groups. The new generation of polymers show outstanding mechanical stability allowing the operation of hundreds of cycles for some enzymatic processes, e.g. antibiotic manufacture.

Epoxy methacrylate resins are offered in different porosities (see Table 2) with the aim to cover a wide range of applications using different enzymes and substrates:

- Lifetech ECR8206 Epoxy methacrylate with small porosity of 500 - 600Å
- Lifetech ECR8204 Epoxy methacrylate with small porosity of 300 - 600Å
- Lifetech ECR8209 Epoxy methacrylate with medium porosity of 600 - 1200Å
- Lifetech ECR8215 Epoxy methacrylate with large porosity of 1200 - 1800Å
- Lifetech ECR8285 Epoxy/butyl methacrylate with porosity of 400 - 600Å

Figure 3 – Immobilization of enzymes on epoxy carriers



Purolite ECR Epoxy resin **Covalently immobilized enzyme**

[1] Lifetech ECR epoxy resins undergo typical degradation of epoxy rings. All Lifetech ECR resins are supplied with clear indication of the expiry date.

All the Epoxy methacrylates mentioned on the previous page are epoxy-activated resins produced with a high degree of crosslinking in the presence of a porogenic agent that allows the precise control of porosity (Table 2). These carriers are stable during storage and easy to handle before, after, and during the immobilization procedures. All epoxy methacrylate resins are designed to form very stable covalent linkages with different protein groups (amino, thiol, phenolic) under very mild pH and temperature conditions (Figure 3). The resins are mechanically very stable and the final immobilized biocatalysts can be used in either a stirred tank or column reactor. Performance of all epoxy methacrylates in the immobilization of enzymes are excellent compared to other commercial products.

Lifetech ECR8206 Epoxy methacrylate has been designed with a very high level of cross-linking for extreme applications like antibiotic manufacture, in which abrasion, mechanical stress and high number of cycles require a very robust resin.

Lifetech ECR8204 Epoxy methacrylate shows outstanding mechanical stability (see Figure 9) and it is manufactured with a lower cross-linking than ECR8206. Lifetech ECR8209 and ECR8215 are designed to offer high porosity with exceptional good mass transfer.

Lifetech ECR8285 is a newly developed epoxy/butyl methacrylic resin. The resin is particularly suitable for the immobilization of lipases and for their use in aqueous/biphasic systems in the presence of hydrophobic substrates. This carrier is unique because it combines epoxy groups for covalent binding with highly hydrophobic matrix thus being optimal for covalent immobilization of lipases, especially CALB.

Table 2 – Applications & technical features for Lifetech ECR epoxy resins[a]

LIFETECH PRODUCT[†]	TYPE	FUNCTIONAL GROUP	IMMOBILIZATION	PORE DIAMETER (Å)
ECR8206	Epoxy methacrylate	Epoxy	Covalent	500 - 600
ECR8204	Epoxy methacrylate	Epoxy	Covalent	300 - 600
ECR8209	Epoxy methacrylate	Epoxy	Covalent	600 - 1200
ECR8215	Epoxy methacrylate	Epoxy	Covalent	1200 - 1800
ECR8285	Epoxy/butyl methacrylate	Epoxy	Covalent	400 - 600

[†] Available as F grade (150 - 300 µm), M grade (300 - 710 µm) and full grade (150 - 710 µm).
[a] Resins are supplied in wet form and do not require any treatment before use

Table 3 – Main properties for Lifetech ECR epoxy resins

LIFETECH PRODUCT[†]	TYPE	MECHANICAL STRENGTH	HYDROPHILICITY	POROSITY
ECR8206	Epoxy methacrylate	• • • •	• • •	• •
ECR8204	Epoxy methacrylate	• • •	• • •	• •
ECR8209	Epoxy methacrylate	• •	• • •	• • •
ECR8215	Epoxy methacrylate	•	• • • •	• • • •
ECR8285	Epoxy/butyl methacrylate	•	•	• •

Table 3 provides some indications for the selection of epoxy carriers.

Amino-activated resins

Another procedure for covalent immobilization of enzymes is based on the use of amino resins. Amino resins can be pre-activated by glutaraldehyde and then used in the covalent immobilization of enzymes (see Figure 4). Reaction of the aldehyde groups with amino groups of enzymes to form *Schiff bases* is fast and gives stable multipoint covalent binding. An even more stable linkage can be achieved by reduction with borohydrides.

Purolite offers different amino resins, with short and long spacer (see Table 4). All amino resins are offered in three different porosities with the aim to cover a wide range of applications using different enzymes and substrates:

- Lifetech ECR8305 Amino C2 methacrylate with small porosity (300 - 600Å)
- Lifetech ECR8309 Amino C2 methacrylate with medium porosity (600 - 1200Å)
- Lifetech ECR8315 Amino C2 methacrylate with large porosity of (1200 - 1800Å)
- Lifetech ECR8404 Amino C6 methacrylate with small porosity of (300 - 600Å)
- Lifetech ECR8409 Amino C6 methacrylate with medium porosity of (600 - 1200Å)
- Lifetech ECR8415 Amino C6 methacrylate with large porosity of (1200 - 1800Å)

Lifetech ECR8305, ECR8309 and ECR8315 Amino C2 methacrylate are amino resins with short ethylene spacer and three different porosities to allocate small and large enzymes.

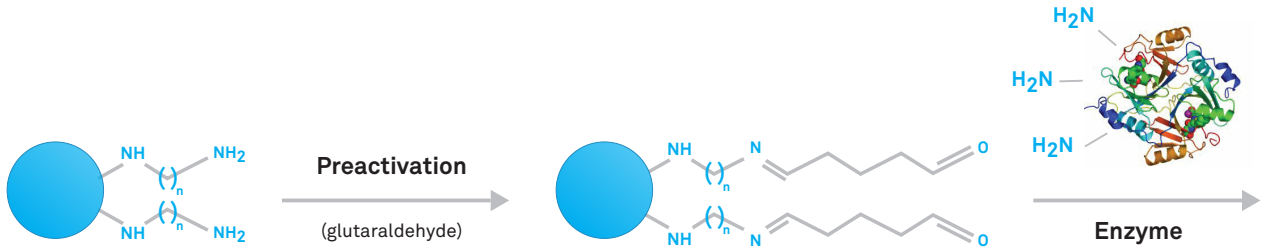
Lifetech ECR8404, ECR8409 and ECR8415 Amino C6 methacrylate are amino resins with a long hexamethylene spacer and three different porosities to allocate small and large enzymes.

Table 4 – Applications & technical features for Lifetech ECR amino C2 methacrylate resins[a]

LIFETECH PRODUCT[†]	TYPE	FUNCTIONAL GROUP	IMMOBILIZATION	PORE DIAMETER (Å)[b]
ECR8305	Amino C2 Methacrylate	NH_2 (short spacer)	Covalent	300 - 600
ECR8309	Amino C2 Methacrylate	NH_2 (short spacer)	Covalent	600 - 1200
ECR8315	Amino C2 Methacrylate	NH_2 (short spacer)	Covalent	1200 - 1800
ECR8404	Amino C6 Methacrylate	NH_2 (long spacer)	Covalent	300 - 600
ECR8409	Amino C6 Methacrylate	NH_2 (long spacer)	Covalent	600 - 1200
ECR8415	Amino C6 Methacrylate	NH_2 (long spacer)	Covalent	1200 - 1800

[†] Available as F grade (150 - 300 µm), M grade (300 - 710 µm) and full grade (150 - 710 µm).
[a] Resins are supplied in wet form and do not require any treatment before use.

Figure 4 – Immobilization of enzymes using amino carriers



Amino resin

n = 2 (short spacer)
n = 6 (long spacer)

Activated resin

Covalently immobilized enzyme
(imino bond formation)

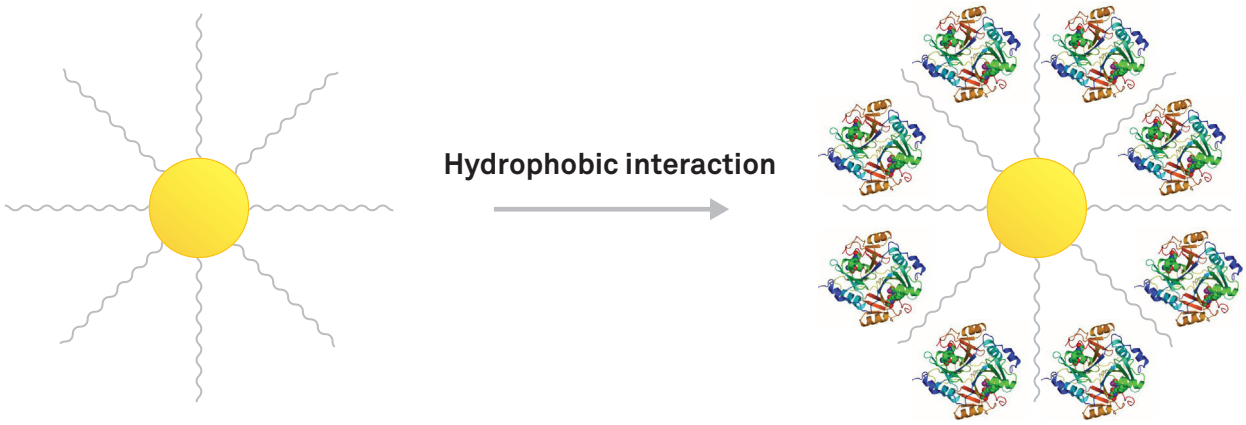
Resins using enzyme adsorption

This method for the immobilization of enzymes is based on the physical adsorption of enzyme on the surface of water-insoluble carriers. The method is very gentle and causes little or no conformational change of the enzyme therefore not affecting its active center. This method is particularly suitable for applications in organic solvents or hydrophobic media such oils. A major advantage of adsorption as a general method of immobilizing enzymes is that usually no reagents are required.

Octadecyl-activated carriers

Octadecyl-activated resins allow reversible but very strong adsorption of enzymes on these highly hydrophobic supports; the enzyme may be desorbed after its inactivation and the support may be reused. Adsorption on octadecyl-activated resins occurs via interfacial activation of the lipase on the hydrophobic supports at very low ionic buffer strength (Figure 5).

Figure 5 – Immobilization of enzymes using octadecyl-activated carriers



Lifetech ECR8804/ECR8806 octadecyl methacrylate are octadecyl (C18) activated resins produced by a very intense crosslinking process in the presence of a porogenic agent that allows the control of porosity (350 - 450Å and 500 - 700Å respectively, see Table 5). Because of the optimized number of octadecyl groups on the matrix, the performance of Lifetech ECR8804 and Lifetech ECR8806 octadecyl methacrylate in the immobilization of enzymes is excellent compared to other commercial products, and the immobilized biocatalyst can be used in organic solvent. The mechanical stability of these resins is very good allowing the final immobilized biocatalysts to be used in both stirred tank and column reactors.

Table 5 – Applications & technical features for Lifetech ECR octadecyl resins

LIFETECH PRODUCT[†]	TYPE	FUNCTIONAL GROUP	IMMOBILIZATION	SURFACE AREA (m^2/g)[a]	PORE DIAMETER (Å)[b]	WATER CONTENT (%)[c]
ECR8804	Octadecyl methacrylate	Octadecyl	Adsorption	N/A	350 - 450	45 - 50
ECR8806	Octadecyl methacrylate	Octadecyl	Adsorption	> 80	500 - 700	58 - 63

[†] Available as F grade (150 - 300 µm), M grade (300 - 710 µm) and full grade (150 - 710 µm).
[a] Determined by B.E.T.
[b] Determined by Hg intrusion.
[c] Resins are supplied in wet form and do not require any treatment before use.

Macroporous carriers

Lifetech ECR1090 and Lifetech ECR1091 macroporous styrene have a high degree of hydrophobicity and are optimal for the immobilization of lipases (Table 6). These resins are stable during storage and can easily be handled before and after immobilization procedures (Figure 6). Lifetech ECR1090 and Lifetech ECR1091 macroporous styrene are produced with high degree of crosslinking in the presence of a porogenic agent that allows the calibration of porosity. These products have a porosity of 900 - 1100Å and 950 - 1200Å (pore diameter) and very high surface area compared to other ECR resins that facilitate mass transfer. Lifetech ECR1090 and Lifetech ECR1091 macroporous styrene are very stable mechanically and the final immobilized biocatalysts can be used in both stirred tank and column reactors. ECR1030M is a robust styrene/methacrylic enzyme carrier for immobilization of lipases by adsorption suitable for batch or column reactors.

Figure 6 – Immobilization of enzymes on macroporous carriers

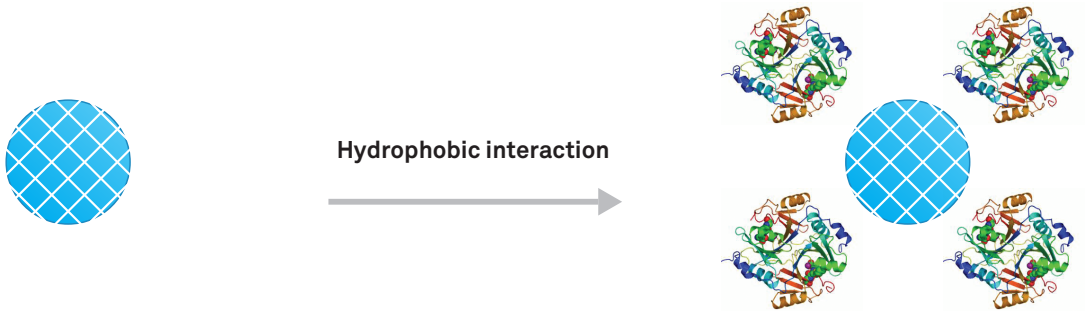


Table 6 – Applications & technical features for Lifetech ECR macroporous styrene resins

LIFETECH PRODUCT[†]	TYPE	FUNCTIONAL GROUP	IMMOBILIZATION	SURFACE AREA (m^2/g)[a]	PORE DIAMETER (Å)[b]	WATER CONTENT (%)[c]
ECR1090	Macroporous styrene	None	Adsorption	> 750	900 - 1100	67 - 73
ECR1091	Macroporous styrene	None	Adsorption	> 450	950 - 1200	62 - 68
ECR1061M[‡]	DVB/ Methacrylate	None	Adsorption	> 400	600 - 750	60 - 70
ECR1030M[‡]	DVB/ Methacrylate	None	Adsorption	> 90	200 - 300	57 - 63

[†] Available as F grade (150 - 300 µm), M grade (300 - 710 µm) and full grade (150 - 710 µm).
[‡] Lifetech ECR1030M and ECR1061 are available only in M grade (300 - 710 µm) and full grade (150 - 710 µm).
[a] Determined by B.E.T.
[b] Determined by Hg intrusion.
[c] Resins are supplied in wet form and do not require any treatment before use.

Resins using ionic immobilization of enzymes

Lifetech ECR resins for ionic enzyme immobilization are macroporous polystyrenic weak or strong base anion resins having tertiary amine or quaternary amine functionality (Table 7). Lifetech ECR resins for ionic enzyme immobilization have an industrial particle size grade that allows easy scale-up.

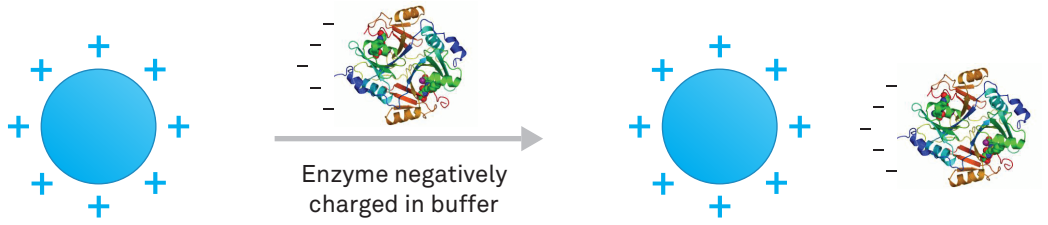
Ionic immobilization is the simplest of all immobilization techniques and does not grossly alter the activity of the bound enzyme. In case of enzymes immobilized through ionic interactions, adsorption and desorption of the enzyme depends on the basicity of the ion exchanger. The dynamic equilibrium between the enzyme and the support depends on the isoelectric point of the enzyme, its optimal pH of activity and the ionic strength of the immobilization buffer. Reversible binding is exploited to enable the economic recovery/regeneration of the support after enzyme activity exhaustion. Ionic immobilization of enzymes has been successfully adapted in many industrial food processes.

Lifetech ECR resins for ionic enzyme immobilization can be applied to immobilization of various enzymes including:

- Invertase
- Glucosyltransferase
- Lipase RM
- Glucoamylase
- Glactosidase

Lifetech ECR1504 and Lifetech ECR1508 are styrene based resins functionalized with different levels (or amount) of tertiary amines. Lifetech ECR1604 and Lifetech ECR1640 are styrene based resins functionalized with different levels of quaternary amines. Before immobilization, resins are equilibrated in HCl and are positively charged. Lifetech ECR resins for ionic immobilization, are fully stable to temperatures up to 100°C. Typical pH for immobilization is in the range 4-5, close to the pI for many glycosidases. Ionic immobilization happens between the positive charges of the resins and the negative charges present on the surface of the enzyme. Ionic immobilization is suitable for large-scale food production applications and Purolite offers Lifetech ECR resins for ionic immobilization for industrial use. (Mean particle size 300 - 1200 µm).

Figure 7 – Immobilization using Lifetech ECR enzyme carriers for ionic enzyme immobilization



Charged resins

Immobilized enzyme through ionic interaction

Table 7 – Lifetech ECR enzyme carriers for ionic enzyme immobilization					
LIFETECH PRODUCT†	TYPE	FUNCTIONAL GROUP	IMMOBILIZATION	TOTAL CAPACITY (eq/mL)	MOISTURE (%)
ECR1504	Styrene tertiary amine	$-NR_2$	Ionic	1.3	53 - 62
ECR1508	Styrene tertiary amine	$-NR_2$	Ionic	1.5	51 - 58
ECR1604	Styrene quaternary amine	$-NR_3^+$	Ionic	1.15	53 - 58
ECR1640	Styrene quaternary amine	$-NR_3^+$	Ionic	0.85	66 - 72

† Particle size: 300 - 1200 µm (industrial grade).
* Temperature stability: all resins are stable up to 100°C in their Cl^- form. Resin can be provided at optimal pH for immobilization in their Cl^- form. Typical pH for immobilization is in the range 4-5.

Particle size, packaging and available kits

All Lifetech ECR resins are available with the following mean particle sizes:

- F Grade: 150 - 300 µm
- M Grade: 300 - 710 µm
- Full Grade: 150 - 710 µm
- Industrial Grade: 300 - 1200 µm for use in ionic enzyme immobilization

Lifetech ECR1030 and ECR1061 are available only as M grade and Industrial Grade.

Standard packing is 50g, 250g, 1kg and 5kg. Larger packing is in 25kg and 50kg. Resins are supplied in wet form and do not require cleaning or washing pretreatment before use.

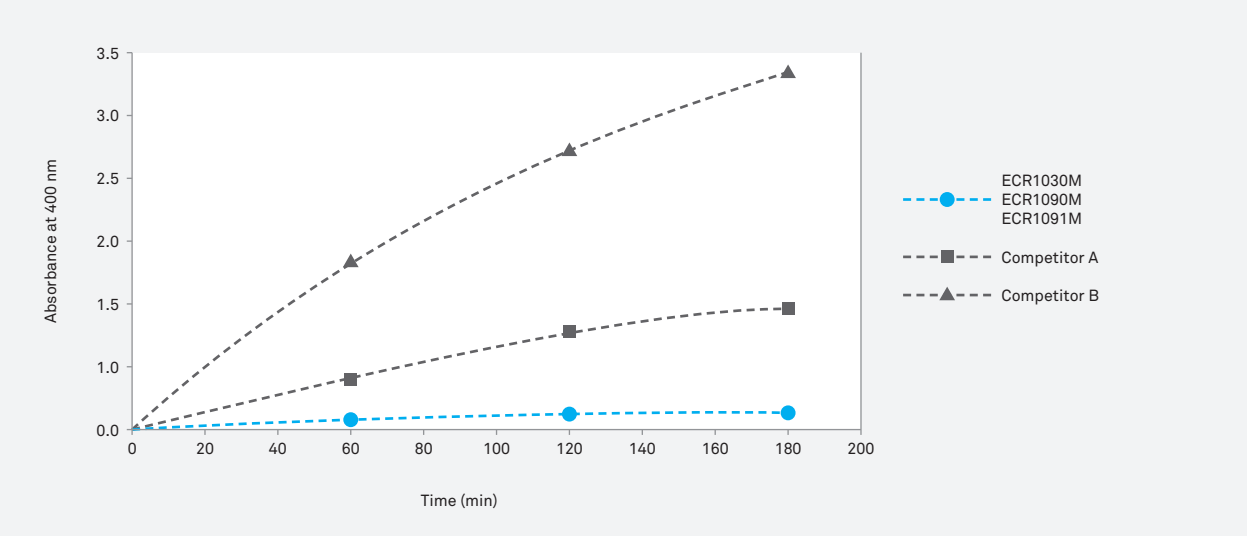
Lifetech ECRKIT1 enzyme carrier kit is designed for screening purposes with sample sizes of 50g (Table 8).

Table 8 – Content of Lifetech ECRKIT1 enzyme carrier kit		
LIFETECH PRODUCT	FUNCTIONAL GROUP	IMMOBILIZATION
ECR8204F Epoxy methacrylate	Epoxy	Covalent (hydrophilic)
ECR8285M Epoxy/butyl methacrylate	Epoxy	Covalent (hydrophobic)
ECR8309F Amino C2 methacrylate	NH_2 (short spacer)	Covalent (hydrophilic) or ionic
ECR8806F Octadecyl methacrylate	Octadecyl	Adsorption
ECR1090F Macroporous styrene	None	Adsorption
ECR1030M DVB/Methacrylate	None	Adsorption

Mechanical stability of Lifetech ECR resins

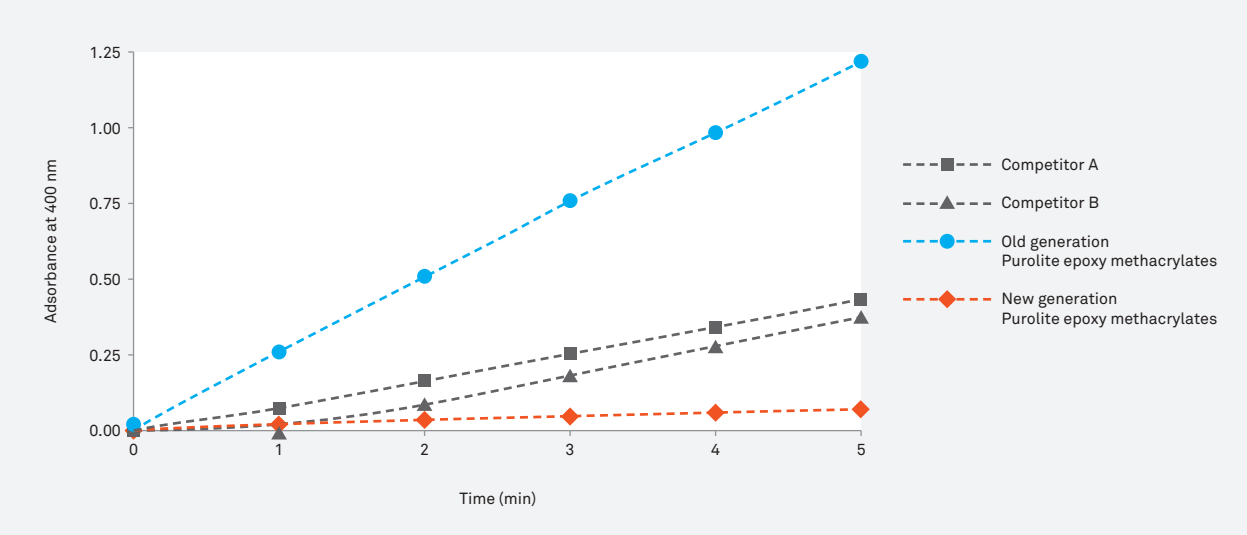
Lifetech ECR resins show outstanding mechanical stability compared to other commercial resins. Mechanical stability is measured by magnetic stirring tests. Resin is added to demineralized water in a glass beaker and mixed using a magnetic stirrer for a period of 3 hours at room temperature. Samples from the solution were analyzed at various time intervals and the absorbance measured at 400 nm. The increase in the absorbance is related to the formation of particulates in the water due to the resin beads breaking.

Figure 8 – Mechanical stability of Lifetech ECR resins for enzyme adsorption



methacrylate from Purolite. Competitor A and B are commercially available epoxy methacrylates in the same particle size.

Figure 9 – Mechanical stability of Lifetech ECR for covalent enzyme immobilization



Lifetech ECR1030M robust divinyl benzene/methacrylate polymer for immobilization of lipase CALB

Candida antarctica lipase B (CALB) is the most versatile biocatalyst and is widely used in industry. Its advantages range from thermostability, stereoselectivity and activity in organic solvents to the acceptance of nucleophiles other than water. The actual standard for industrial applications of CALB is the immobilized enzyme on Lewatit® VP OC 1600 and is marketed under the name Novozym® 435. A new methacrylic enzyme carrier (Lifetech ECR1030M) for the immobilization by adsorption of CALB and for use in batch or column reactors has been developed (Table 9). The resin allows a TYPICAL activity of > 10000 PLU/g_{dry}, which is similar to Novozym® 435. With a protein loading of 24.4 mg/g_{wet} it is possible to obtain immobilizations yields higher than 90%. Unlike the resin used for the production of Novozym® 435, Lifetech ECR1030M possesses high mechanical stability and is therefore more suitable for applications that expose the polymers to high mechanical stress, such as multiple reuses in stirred reactors.

Table 9 – Properties of Lifetech ECR1030M and Lewatit VP OC 1600

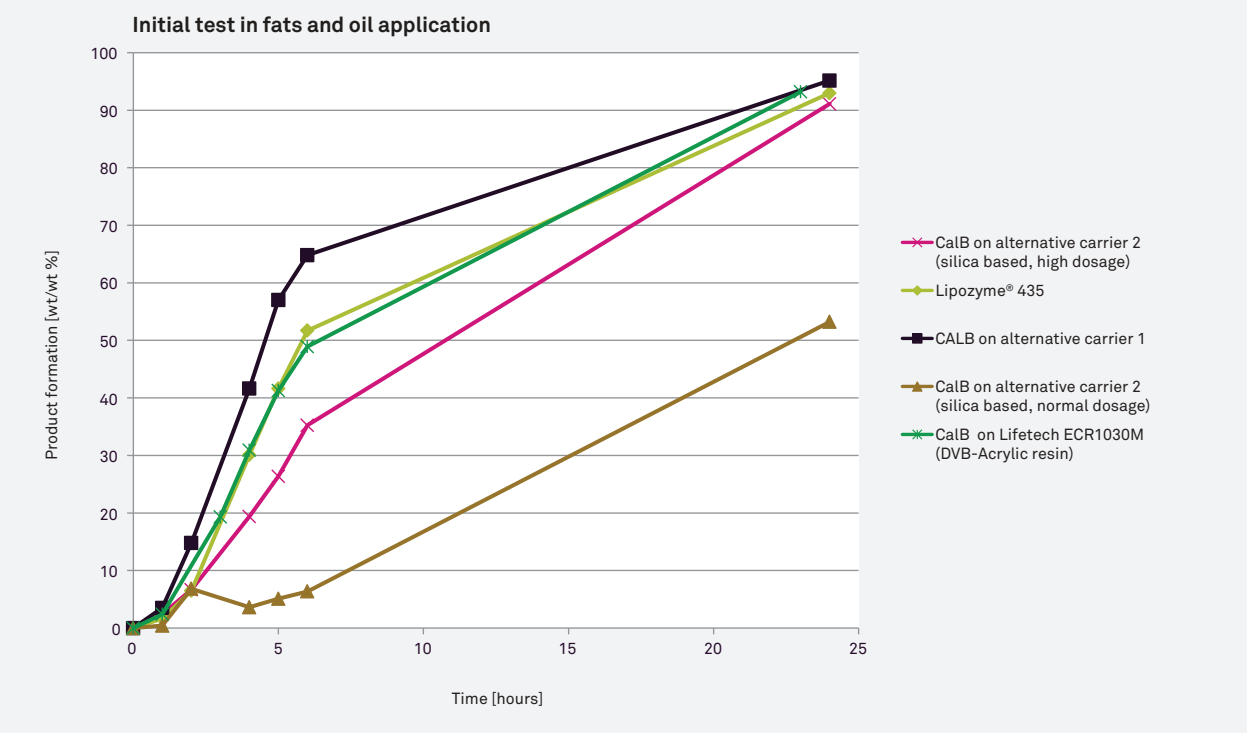
CARRIER	PARTICLE SIZE (micron)	POROSITY* (Å)	SURFACE AREA (m^2/g)	PORE VOLUME (mL/g)†	ACTIVITY (PLU/g_{dry})‡
ECR1030M	300 - 710	250	75	0.39	> 9000
Lewatit VP 1600 OC	315 - 1000	324	74	0.51	> 9000

* Determined by mercury intrusion.
† Determined by mercury intrusion.
‡ Enzymatic activity obtained by immobilization of 25KU of Lipase CALB per gram dry. Activity measure by propyl laurate synthesis.

A. Basso, L. Froment, M. Hesseler, S. Serban, Eur. J. Lipid Sci. Technol. 2013, 115, 468-472.

Lipase CALB immobilized on Lifetech ECR1030M shows excellent performance in transesterification of vegetable oil and shows the same kinetics as Lipozyme® 435 (see Figure 10).

Figure 10 – Initial test of CALB preparations immobilized on carriers for the transesterification of vegetable oil



Courtesy of Novozymes

References

The following list contains references to literature recommended for further reading on the topic of enzyme immobilization.

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For detailed immobilization procedures, please download the Lifetech ECR Enzyme Immobilization Procedures application guide available on www.purolitelifesciences.com

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