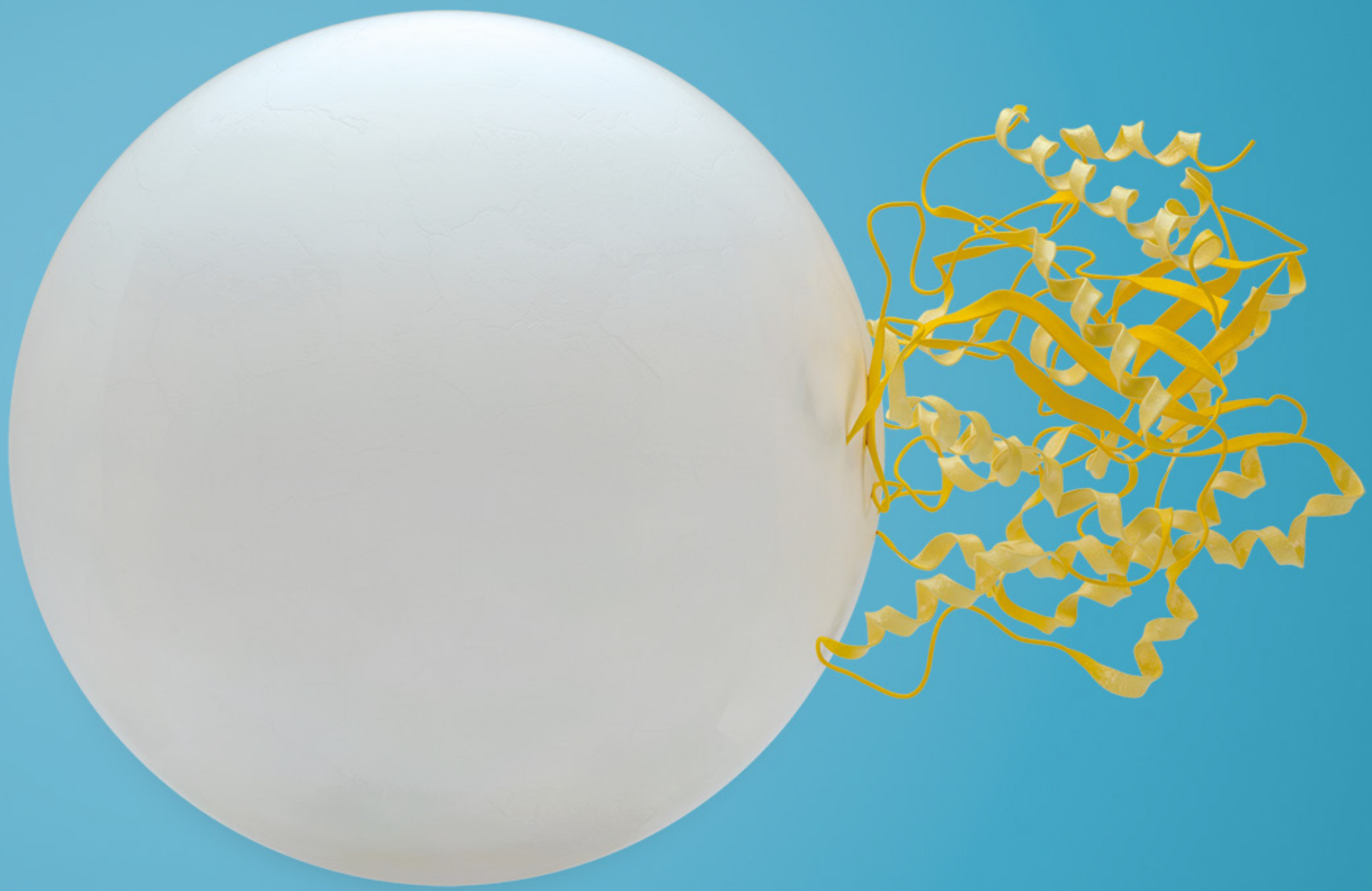
Enzyme Immobilization Resins



Lifetech™ ECR

for ionic, covalent or adsorption-based enzyme immobilization

Chromalite® MIDA

for affinity-based (His-tag) enzyme immobilization and purification

Purolite®

Since 1981, Purolite® has grown into the world's premier resin-based separation, purification and extraction technology manufacturer and innovation leader, with manufacturing facilities, advanced research laboratories and over 1100 people employed world-wide.

Purolite® brings innovative thinking and a distinguished history of resin technology expertise to the global Life Sciences marketplace.

We provide APIs, enzyme carriers, immobilized enzymes, and chromatographic resins of the highest quality, to support research and development and production-scale applications in global pharmaceutical, food and beverage, bioprocessing, cosmetics and fine chemical markets.

Quality

Purolite® maintains a global Quality Management System (QMS) which supports BSI requirements of ISO 9001. Compliance is monitored and maintained through a quality assurance and regulatory team, who conduct internal audits to ensure operations meet the guidelines and protocols for equipment and procedures. Our teams are given continuous training on quality processes to ensure batch-to-batch consistency, and the highest product quality.

Secure supply through manufacturing excellence

Ensuring reliable availability of our resins is vital to customers, and of paramount importance to Purolite®. As a leading supplier of resin to the world's most regulated industries, we recognise that our resins are critical purification products.
As such, a real-world security-of-supply system is in place to support your process requirements for business continuity.

Supply risk is managed end-to-end, with a global network of qualified suppliers. Long-term supply agreements with periodic audits ensure consistency and 'fit for purpose' performance.

Purolite® has manufacturing facilities at 4 strategic locations in the USA, Asia and Europe. Lifetech™ and Chromalite® products are manufactured at both UK and Romania-based facilities, which are fully certified under ISO 9001:2015, ISO 14001:2015 and ISO 45001:2018. Our Romanian facility is FDA-inspected, with a total of 4 state-of-the-art clean rooms, offering separate facilities for ligand/enzyme immobilization, removal of fines, solvent or purified water washing, screening, vacuum drying and packaging.

Lifetech™ ECR resins are manufactured via traditional suspension polymerization techniques and activation chemistries at each production scale depending on volume required. At our Romanian production site, Clean Room 4 (CR4) is fully equipped with a wet screener, a reactor for resin functionalization and equipment for the drying and packing of products in a controlled environment. The processing capacity of CR4 is greater than 1 ton of resin per batch.

100% focused on resin technology.

Global manufacturing at facilities in the UK, Romania, China and USA.

ISO-certified, state-of-the-art manufacturing facilities

30+ years of regulatory experience from FDA inspected cGMP facility.

40+ years of experience in solving advanced R&D and purification challenges.

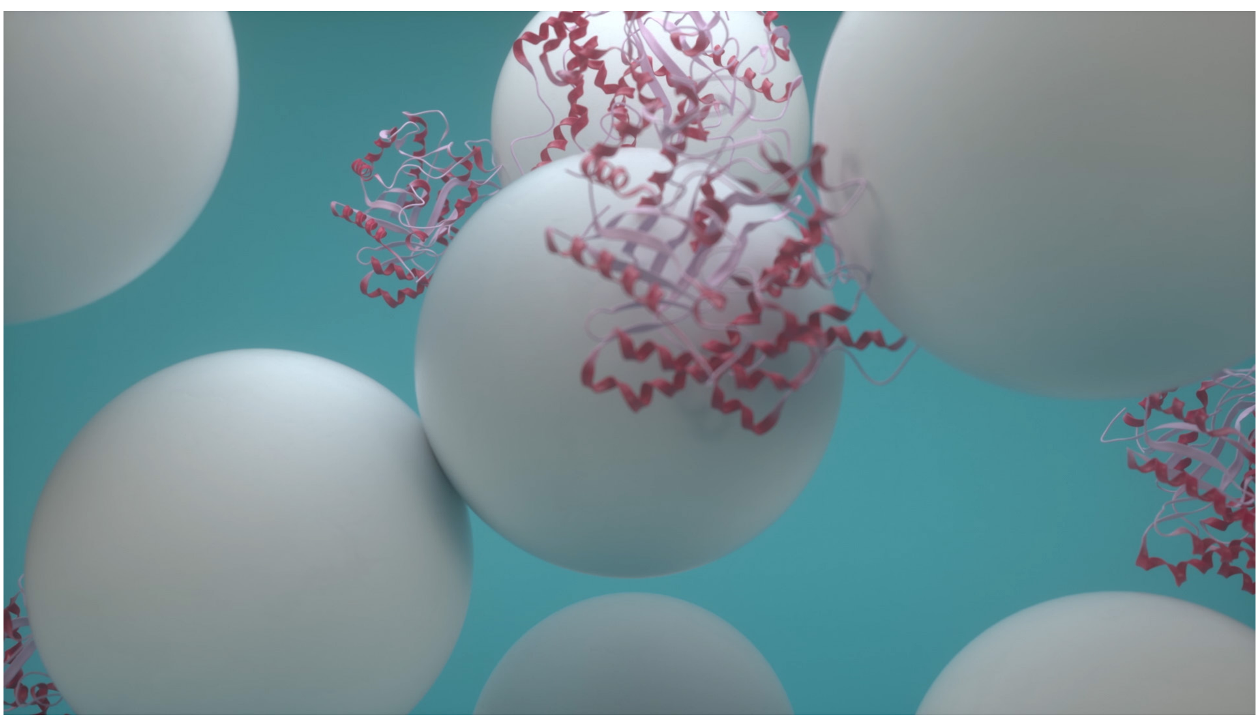
Enzyme immobilization resins for research and industrial biocatalysis

The Lifetech™ ECR range is the single largest portfolio of enzyme immobilization resins in the world. With styrenic and methacrylic base matrices with a wide range of physical, chemical and mechanical properties, we have the solution you need for efficient ionic, covalent or adsoption-based enzyme immobilizations in pharmaceutical, chemical, nutraceutical or food and beverage applications.

High cross-linking ensures mechanical stability, and high functional group density allows multipoint covalent binding for minimal enzyme leakage. Immobilization via ionic interaction is achieved with weak base anion exchange resins which are cost-effective, as they can be regenerated after enzyme exhaustion.

For affinity-based enzyme immobilizations, our Chromalite® MIDA range offers high His-tagged enzyme selectivity and stability, sometimes more flexible than other immobilization techniques. Porosities typically in the range of 1000Å, make Chromalite® MIDA ideal for immobilizing a wide variety of enzymes.

This document provides comprehensive technical and sales information, and is designed to aid you in selecting your optimal product.



Contents

Immobilized enzymes - an introduction

Immobilized enzymes are powerful process optimization tools in both operative and economic terms.

Today, immobilized enzymes are used as biocatalysts in the production of vital pharmaceuticals, chemicals and food additives at a variety of scales, from just a few kilograms to multiple tons. Some of these industrial processes are listed in Table 1.

Applications using immobilized enzymes offer a high degree of flexibility, as they are suitable for either continuous processes (using fixed or expanded-bed reactors) or in batch processes (using stirred-tank configurations).

Three primary benefits of immobilized enzymes are:

- Simple separation of the biocatalyst from the product
- Re-use of the biocatalyst
- More convenient handling of enzyme preparations

There are also three primary critical parameters in the preparation and use of immobilized biocatalysts, which have a significant impact on the efficacy of the system and must be tightly controlled. These critical parameters are:

- Immobilization yield
- Mass transfer limitations
- Operational stability

The immobilization of the enzyme on a rigid structure increases the stability of the biocatalyst - especially when utilizing organic solvents - by preventing the protein from unfolding to a certain degree.

Operative binding forces vary between weak multiple adsorptive interactions and attachments through strong covalent binding. The most appropriate immobilization method to use is usually determined by both the application parameters and the specific type of enzyme which is to be immobilized.

TABLE 1 – Examples of immobilized enzymes in industrial applications

Enzyme	Substrate	Product	Amount (tons/year)	Application
Aminoacylase	Acyl-D-L-amino acid	L-Amino acid	300	Food
Aspartase	Fumaric acid	L-Aspartic acid	1,200	Chemicals
Aspartase β-decarboxylase	Aspartic acid	L-Alanine	120	Chemicals
Cephalosporin amidase	Glutaryl-7-ACA	7-ACA	Unknown	Pharmaceutical
Fumarase	Fumaric acid	L-Malic acid	360	Chemicals
Glucose isomerase	Glucose	HFCS	8,000,000	Food
Lactase (galactosidase)	Lactose	Lactose free milk	Unknown	Dairy
Lipase	Rac-1-phenylethylamine	S-1-Phenylethyamine	200	Chemicals & food
Nitrile hydratase	Acrylonitrile	Acrylamide	30,000	Chemical & wastewater treatment
Penicillin amidase	Penicillin G	6-APA	6,000	Pharmaceutical

Which key parameters affect immobilized enzyme performance?

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

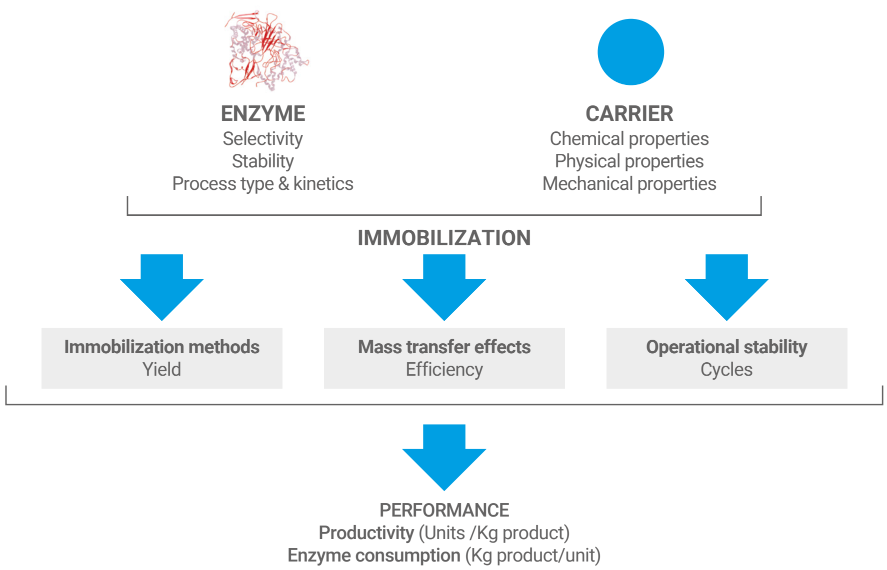
Where manufacturing costs are concerned, the yield of immobilized enzyme activity is mostly determined by the immobilization method and the amount of soluble enzyme used. Under process conditions, the resulting activity may be further reduced by mass transfer effects. More precisely, the yield of enzyme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

Conversely, improved stability under operating conditions, and additional re-uses of the enzyme 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 we replace “enzyme units” with “enzyme costs” we obtain the essential product-related costs per kg of product.

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 (Figure 1).

FIGURE 1

Parameters affecting the performance of immobilized enzymes



Important enzyme carrier properties

Functional group of the resin

The type of activation, presence, distribution and density of functional groups determines the activity yield of an immobilization reaction, the stability and operational stability of the carrier-fixed enzyme. Lifetech™ ECR resins are offered in a variety of functional groups with varying porosities and hydrophobicities.

Porosity and surface area

In most cases, controlled surface area and large pore diameter are desirable so that both enzyme and substrate can be easily penetrated. A pore size of >300 Å is usually adequate to make the internal surface accessible to the majority of enzymes. Lifetech™ ECR resins are available with differing degrees of average pore diameter and surface area, usually higher than 400 Å and >20 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/divinylbenzene (DVB), methacrylate or DVB/methacrylate, offering a full range of hydrophobicity to cover any application.

Mechanical stability

These properties are dependent on the type of reactor being used. If used in a stirred tank reactor, the support should be stable against sheer forces to minimize abrasion. Production of fines (usually particles below 50 µm) during usage can lead to the obstruction of sieve plates and filters, as well as contamination of final product. Lifetech™ ECR resins are designed to be mechanically stable, allowing their use in repeated cycles.

Form and size of support

Particle size influences filtration times 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 multi kilo scale up to hundreds of kilos, spherical particles in the range of 150 – 300 µm (such as F grade Lifetech™ ECR resins and C grade Chromalite® MIDA resin) are preferred, as they provide the highest activity and optimal filtration time. For column reactors, where quantities of immobilized enzymes range from hundreds of kilos to several tons, a particle size of 300 – 710 µm (such as M grade Lifetech™ ECR resins) is preferable to avoid high back pressures.

Insolubility

Insolubility 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 spherical beads and can be used for applications in batch or bed column reactors. They are stable in a variety of organic solvents.

Resistance to microbial attack

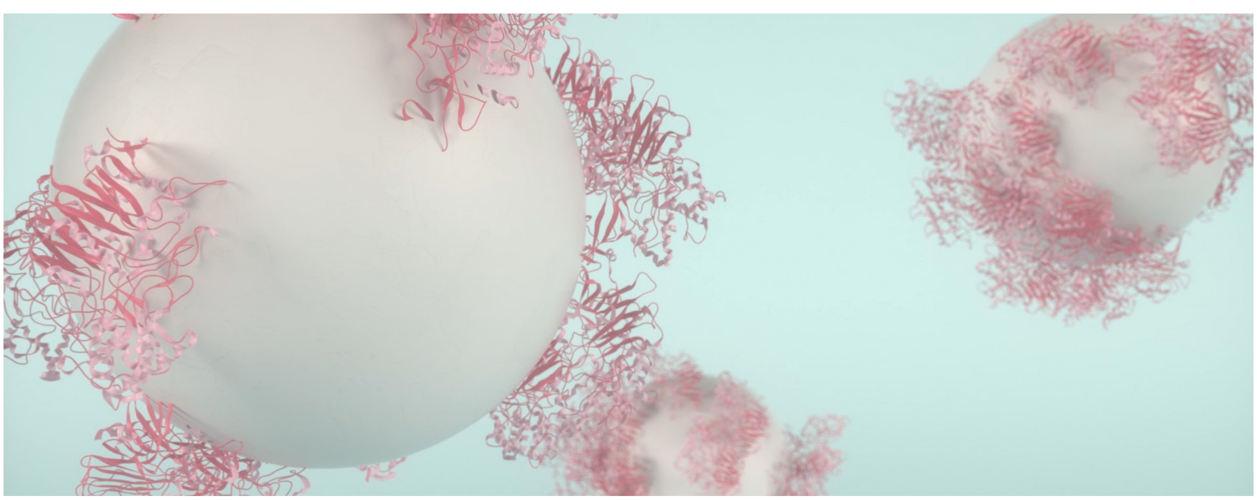
During long-term usage, the support has to be stable against microbial degradation. Lifetech™ ECR resins are inert materials and thanks to the high quality of water used in all Purolite® manufacturing facilities they can be stored for long periods without any loss of performance.

Regeneration

This property is of interest especially when carrier materials are expensive or for specific applications where cost-efficiencies are vital. LifetechTM ECR resins and Chromalite® MIDA resins for affinity immobilizations, adsorption and ionic immobilization can be regenerated and re-used for further enzyme immobilization.

Safety and regulations

Lifetech™ ECR products comply with the Council of Europe Resolution ResAP (2004) 3 on ion exchange and adsorbent resins used in the processing of food materials. They also comply with Halal and Kosher regulations.



Lifetech™ ECR resins and Chromalite® MIDA Resins

Purolite resins for enzyme immobilization include methacrylic or styrenic options, with differing degrees of hydrophobicity and porosity, purpose-designed for enzymes with varying hydrophobicities and sizes.

The full range of functional groups available with Lifetech ECR resins can be seen in Figure 2 and Table 2.

If you require assistance in selecting the optimal product, we have two Screening KITs available, each containing 6 different samples for quick and easy testing.

- The Lifetech™ ECR KIT1 includes six 50g resin samples for rapid product screening of ionic, covalent or adsoption-based enzyme immobilizations (Table 3).
- The SpectraChrom screening KIT for affinity (His-tag) immobilization includes six 25 ml samples of Chromalite® MIDA resin pre-loaded with Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} or Fe^{3+}, as well as Chromalite® MIDA without metal loaded (Table 8).

FIGURE 2

Available Lifetech™ and Chromalite® chemistries for enzyme immobilization

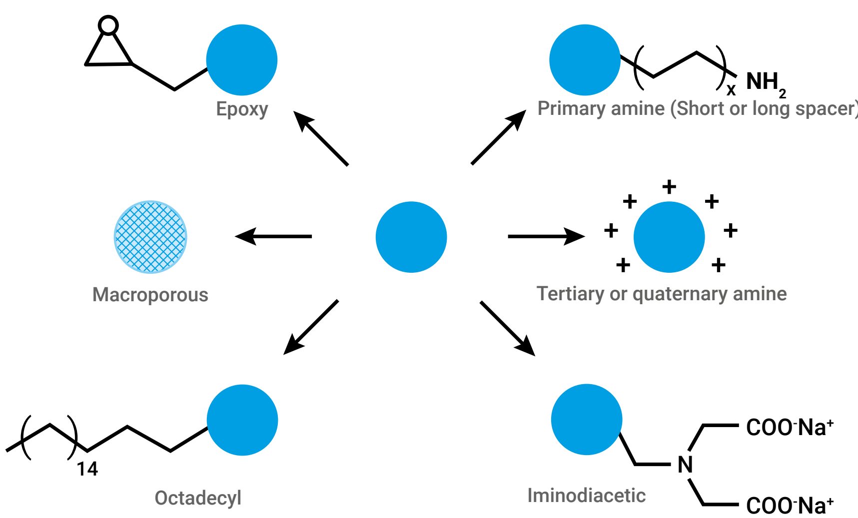


TABLE 2

Immobilization types dependant on functional group (chemistries) of Lifetech™ ECR resins

Functional group	Immobilization type
Epoxy	Covalent
Amino (short or long spacer)	Covalent via pre-activation with glutaraldehyde
Amino (short or long spacer)	Ionic interaction via pre-equilibration to controlled pH
Octadecyl	Adsorption via hydrophobic interaction
Macroporous	Adsorption via hydrophobic interaction
Iminodiacetic	Affinity via His-tag interaction with metal (Ni^{2+}, Co^{2+}, Fe^{3+} , Cu^{2+} or Zn^{2+})

TABLE 3

Contents of Lifetech™ECR KIT1

Kit content	Matrix	Functional group	Immobilization
ECR8204F	Epoxy methacrylate	Epoxy	Covalent (hydrophilic)
ECR8285	Epoxy/butyl methacrylate	Epoxy	Covalent (hydrophobic)
ECR8309F	Amino C2 methacrylate	NH_2 (short spacer)	Covalent (hydrophilic) or ionic/adsorption
ECR8806M	Octadecyl methacrylate	Octadecyl	Adsorption
ECR1090M	Macroporous styrene	None	Adsorption
ECR1030M	DVB/methacrylate	None	Adsorption

Lifetech™ ECR epoxy-functionalized resins

For easy multipoint covalent immobilization of an enzyme on to the resin Lifetech™ ECR epoxy methacrylate resins are offered in a range of porosities (Table 4), covering a variety of applications involving different sizes of enzyme and substrates. All epoxy methacrylate products are produced via crosslinking in the presence of a porogenic agent. This allows full control over the porosity of the resin.

These carriers are easy to handle before, during and after the immobilization procedure, and are designed to form very stable covalent linkages with different protein groups (thiol, amino, carboxylic, phenolic) under very mild experimental conditions of pH and temperature (Figure 3). The resins are available with differing degrees of mechanical stability and the final immobilized biocatalysts can be used in either stirred tank or packed bed reactors (Table 5). The performance of all epoxy methacrylates in the immobilization of enzymes is excellent compared to other commercial products.

Lifetech™ ECR8204 shows outstanding mechanical stability (Figure 14) when compared to other commercial products and is optimal for penicillin amidase immobilization used in antibiotic manufacture, namely 6-APA and synthesis of amoxicillin.

Lifetech™ ECR8285 is a unique epoxy/butyl methacrylic resin. It 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 a highly hydrophobic matrix thus being optimal for covalent immobilization of lipases, such as CalB.

Note: ECR epoxy resins undergo typical degradation of epoxy rings. Lifetech™ ECR resins are supplied with expiry dates.

FIGURE 3

Immobilization of enzymes on epoxy-functionalized carriers

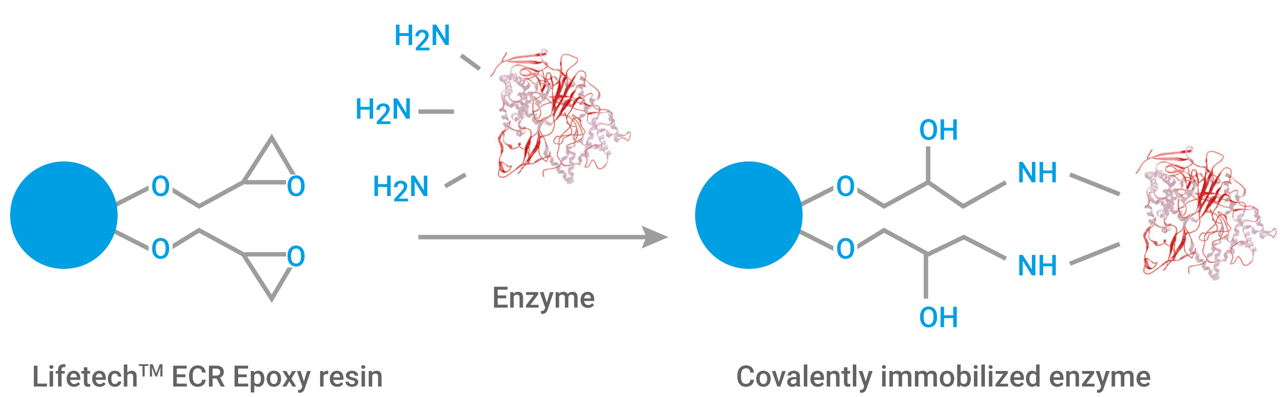


TABLE 4

Lifetech™ ECR resins with epoxy functional groups

Product	Type	Functional group	Immobilization	Pore diameter (Å)[a]
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

[a]: *determined by mercury intrusion*

Note: All resins are available in 2 particle size ranges: F grade (150 - 300 µm) and M grade (300 - 710 µm)

TABLE 5

Key properties of Lifetech™ ECR epoxy resins

Product	Matrix	Mechanical strength	Hydrophilicity	Porosity	Recommended reactor
ECR8204	Epoxy methacrylate	+ + +	+ + +	+ +	Batch /column
ECR8209	Epoxy methacrylate	+ +	+ + +	+ + +	Batch /column
ECR8215	Epoxy methacrylate	+	+ + + +	+ + + +	Column
ECR8285	Epoxy/butyl methacrylate	+	+	+ +	Column

Lifetech™ ECR amino-functionalized resins for covalent enzyme immobilization

Lifetech™ ECR methacrylate resins functionalized with primary amines provide options for covalent enzyme immobilization. Amino resins can be used for immobilization of enzymes using two different methods:

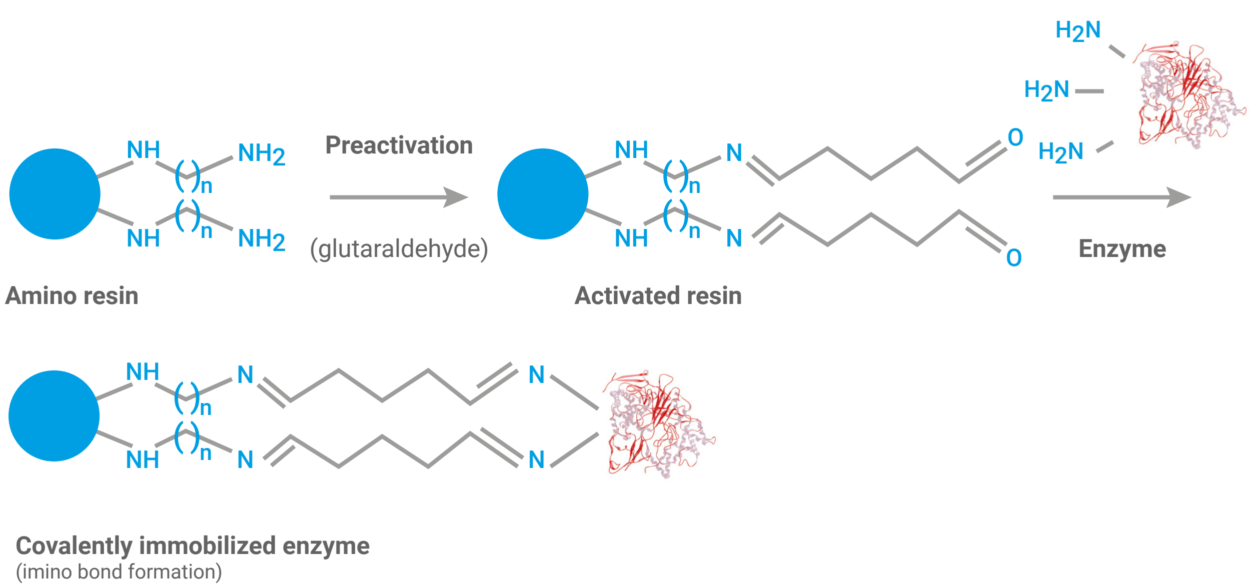
- covalent immobilization by pre-activation with glutaraldehyde (Figure 4)
- ionic interaction by pre-equilibration of the primary amine to specific pH (Figure 5)

Covalent immobilization via Schiff bases:

Reaction of the aldehyde groups with amino groups of enzymes forms Schiff bases which are stable in a pH range 3-8. The available aldehyde groups on the resin provide multipoint covalent binding with terminal amino groups present in the protein.

FIGURE 4

Covalent immobilization of enzymes onto amino-functionalized carriers



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

The Lifetech™ ECR range includes different amino resins, with short and long spacers (Table 6). All amino resins are offered in 3 different porosities with the aim to cover a wide range of applications using different enzymes and substrates.

Short spacer amino resins

Lifetech™ ECR8304, ECR8309 and ECR8315 are amino methacrylic resins with short ethylene (C2) spacers and three different porosities to accommodate small and large enzymes up to 100 kDa.

Long spacer amino resins

Lifetech™ ECR8404, ECR8409 and ECR8415 are amino methacrylic resins with a long hexamethylene (C6) spacer and three different porosities to facilitate the immobilization of small and large enzymes.

TABLE 6

Lifetech™ ECR resins with amino functional groups

Product	Type	Functional group	Immobilization	Pore diameter (Å)[a]
ECR8304	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

[a]: *determined by mercury intrusion*

Note: All resins are available in 2 particle size ranges: F grade (150 - 300 μm) and M grade (300 - 710 μm)

Lifetech™ ECR amino-functionalized resins for ionic enzyme immobilization

Lifetech™ ECR amino-functionalized resins can be used for ionic enzyme immobilization. For this, the recommended particle size is 300 - 710 µm, which is suitable for large industrial columns.

Ionic immobilization is a simple immobilization technique which does not significantly alter the activity of the bound enzyme. When enzymes are immobilized through ionic interactions, adsorption and desorption of the enzyme is dependent on the base of the ion exchanger used (Figure 5). The dynamic equilibrium between the enzyme and the support depends on the isoelectric point of the enzyme, its optimal pH of activity, the ionic strength of the immobilization buffer, the ionic charge of the resin in the immobilization and operating condition.

The ability to reverse the binding allows for the recovery and regeneration of the support after enzyme activity has been exhausted, improving process economics. Ionic immobilization of enzymes has been successfully utilized for many industrial processes for the production of foodstuffs. Lifetech™ ECR resins for ionic enzyme immobilization can be applied to many enzymes including:

- Invertase
- Glucosyltransferase
- Lipase RM
- Glucoamylase
- Galactosidase

Prior to immobilization, resins are equilibrated in a solution at a pH which aims to provide a positive charge to the resin. Ionic immobilization happens between the positive charges of the resin and the negative charges present on the surface of the enzyme at a controlled pH. Ionic immobilization is suitable for large-scale food production applications and Lifetech™ ECR resins for ionic immobilization are available in large quantities.

Lifetech™ ECR8309M/PH4 has shown excellent performance during immobilization of glucoamylase for the production of molasses for the confectionery industry. In this case, the amino resin is already pre-equilibrated at pH 4. Figure 6 shows the principle of glucoamylase immobilization on ECR8309/PH4.

FIGURE 5

Ionic enzyme immobilization on Lifetech™ ECR amino resins

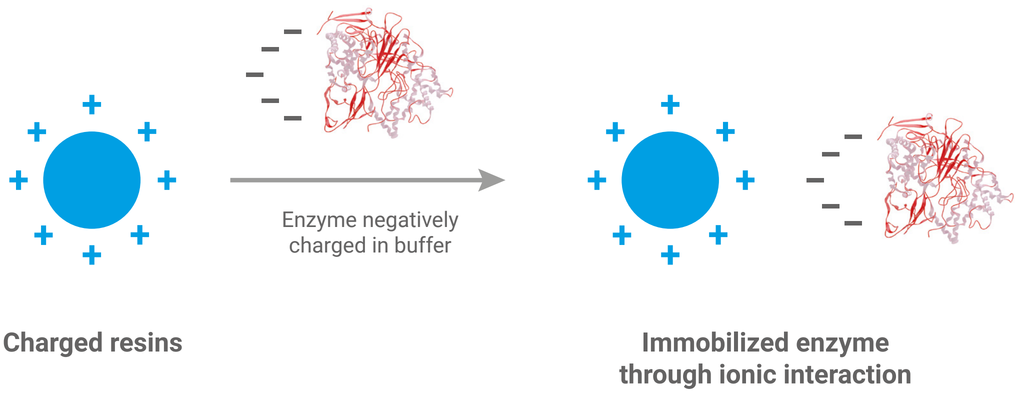
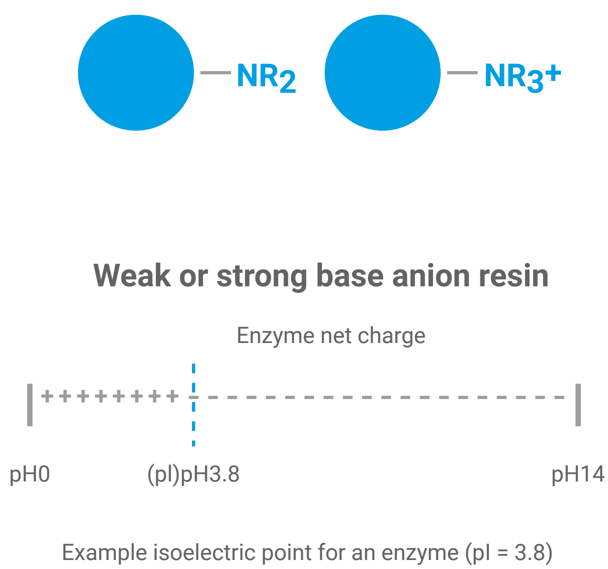


FIGURE 6

Selecting the appropriate operative pH for an enzyme with an isoelectric point of 3.8



Lifetech™ ECR resins for enzyme immobilization by adsorption

Enzyme immobilization by adsorption is based on the physical adsorption of the enzyme on the surface of water-insoluble carriers. The method is very gentle and causes little or no conformational change of the enzyme or destruction of its active center.

This method is particularly suitable for biocatalytic applications in organic solvents or hydrophobic media such as oil. A major advantage of adsorption is that usually no other reagents are required. Lifetech™ ECR resins for immobilization by adsorption offers three different carriers with different polymer backbones and porosities which provide differing degrees of hydrophobicities for interactions with a variety of enzymes (Table 7).

Lifetech™ ECR8806M is an octadecyl-activated resin characterized by high hydrophobicity. This resin allows strong adsorption of enzymes after immobilization and drying. The immobilized enzyme can be used in organic solvent and oils without enzyme leaching. Adsorption on octadecyl-activated resins occurs via interfacial activation of the lipase on the hydrophobic supports at very low ionic buffer strength (Figure 7). The resin is extremely mechanically stable, allowing the final immobilized biocatalysts to be used in both stirred tank and bed reactors.

TABLE 7

Lifetech™ ECR resins for immobilization by adsorption

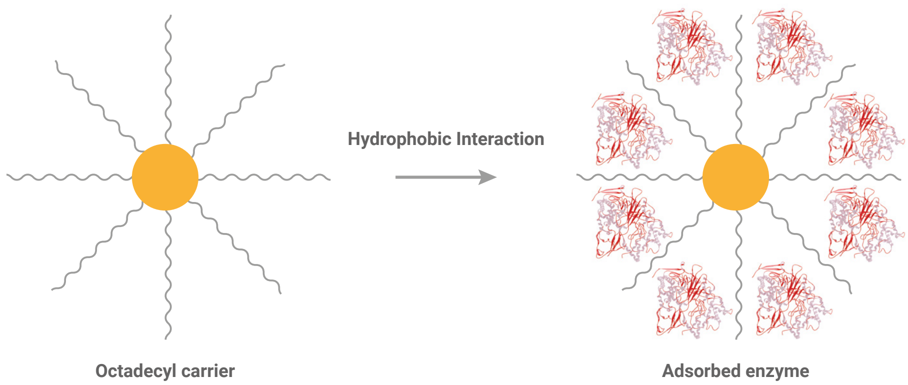
Product	Type	Functional group	Immobilization	Pore diameter (Å)[a]
ECR8806M	Octadecyl methacrylate	Octadecyl	Adsorption	400-650
ECR1030M	DVB/methacrylate	None	Adsorption	220-340
ECR1090M	Macroporous styrene	None	Adsorption	900-1100

[a]: *determined by mercury intrusion*

Note: All resins are available in 2 particle size ranges: F grade (150 - 300 µm) and M grade (300 - 710 µm)

FIGURE 7

Immobilization of enzymes on octadecyl -activated carriers

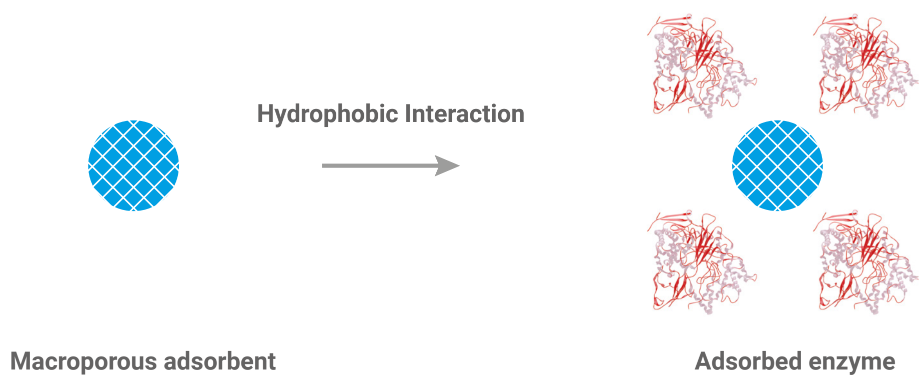


Lifetech™ ECR1090M and ECR1030M have a high degree of hydrophobicity due to the presence of styrene and divinylbenzene (DVB). These resins are optimal for the immobilization of lipases, are stable during storage and can easily be handled before and after immobilization procedures (Figure 8).

These resins are mechanically very stable and the final immobilized biocatalysts can be used in both stirred tank and fixed bed reactors. Lifetech™ ECR1030M is also used for the immobilization of Lipase CalB in our CalB immo Plus™ ready-to-use immobilized enzymes.

FIGURE 8

Immobilization of enzymes by adsorption on macroporous carriers



Chromalite® MIDA resins for affinity immobilization (His-tag technology)

Immobilization of enzymes on solid supports is an excellent solution, and provides significant advantages over some of the industrial processes described earlier in this document. The Purolite® enzyme immobilization resin portfolio also includes resins for immobilization by affinity.

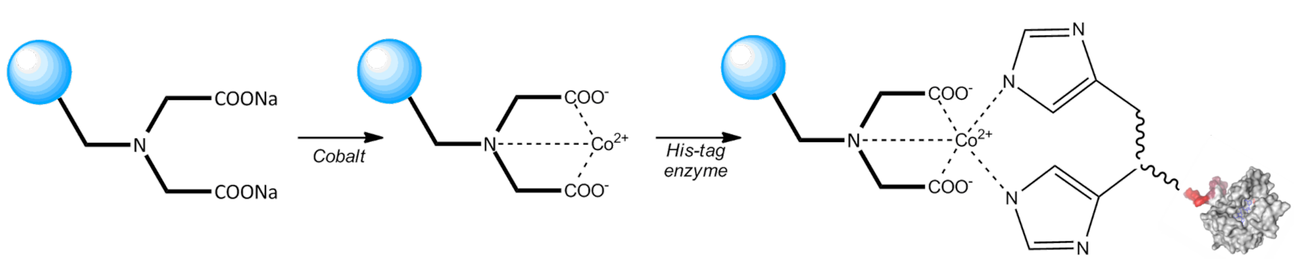
This new range of IDA resins provides high His-tagged enzyme selectivity and stability. Depending on the His-tag position on the protein, this enzyme would be more flexible than other immobilization techniques and can be orientated at the time of immobilization (Figure 9. Additionally, the resin can be regenerated after enzyme activity exhaustion.

To aid you in selecting the optimal Chromalite® MIDA resin product, we have developed the SpectraChrom resin screening KIT.

The KIT includes six 25 ml samples of Chromalite® MIDA resin pre-loaded with Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} or Fe^{3+}, as well as Chromalite® MIDA with no metal loaded. This enables rapid screening during enzyme purification or immobilization. The porosity of Chromalite® MIDA is typically in the range of 1000Å, with particle sizes of 100 - 300 µm making it suitable for the immobilization of a wide variety of enzymes. (Table 8

FIGURE 9

An example of Chromalite® MIDA metal loading and immobilization using cobalt



Chromalite® MIDA   Chromalite® MIDA/Co   Chromalite® MIDA/Co binding to His-tagged enzymes

TABLE 8

Contents of SpectraChrom KIT for immobilization by metal affinity

Product	Description	Loading	Appearance
Chromalite® MIDA/M	Iminodiacetic acid methacrylate	-	White
Chromalite® MIDA/M/Ni	Iminodiacetic acid methacrylate, nickel loaded	>8g/l (Nickel) $NiCl^2$	Green/Blue
Chromalite® MIDA/M/Co	Iminodiacetic acid methacrylate, cobalt loaded	>8g/l (Cobalt) $CoCl^2$	Pink
Chromalite® MIDA/M/Zn	Iminodiacetic acid methacrylate, zinc loaded	>8g/l (Zinc) $ZnCl^2$	White
Chromalite® MIDA/M/Cu	Iminodiacetic acid methacrylate, copper loaded	>8g/l (Copper) $CuCl^2$	Blue
Chromalite® MIDA/M/Fe	Iminodiacetic acid methacrylate, iron loaded	>8g/l (Iron) $FeCl^2$	Orange/Brown

For rapid screening during enzyme purification or immobilization, the SpectraChrom KIT includes six 25 ml samples of Chromalite® MIDA resin pre-loaded with Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} or Fe^{3+}, as well as Chromalite® MIDA with no metal loaded.

Drying of immobilized enzymes

For applications in water-free media such as organic solvents, drying of immobilized enzymes may be required.

Lifetech™ ECR enzyme carriers can be easily dried to achieve minimized water content (<5%). Several important factors must be considered before commencing the drying process for an immobilized enzyme:

- The degree of minimum hydration required by the enzyme. Immobilized lipases, for example, can be dried to <5% with negligible loss of activity.
- The temperature stability of the native enzyme. Such information will provide you with an indication of suitable drying temperature.
- Initial moisture content of the resin after immobilization. A good water removal after immobilization will considerably reduce the drying time. Water removal can be done by filtration under vacuum.

Table 9 summarizes some of the known methods used for the drying of immobilized enzymes, as well as their applications, main advantages and/or disadvantages (Adapted from Perry's Chemical Engineering Handbook,1999).

TABLE 9

Drying processes applicable to immobilized enzymes

Type of dryer	Application / Comment
Fluid bed dryer	• Suitable for small and large scale (mg scale to hundreds of kilos) • Uses dry air or nitrogen to remove the moisture from the resin • Drying can be done at room temperature or higher
Vacuum dryer	• Suitable for pilot scale and industrial scale • Relatively fast drying time with the advantage of low temperatures • Useful for heat sensitive enzymes
(Vacuum) Shelf-dryer	• Suitable for heat sensitive product or readily oxidizable products • Potentially longer drying time • Require large space to process a large amount of product

Common bioreactor configurations when using immobilized enzymes

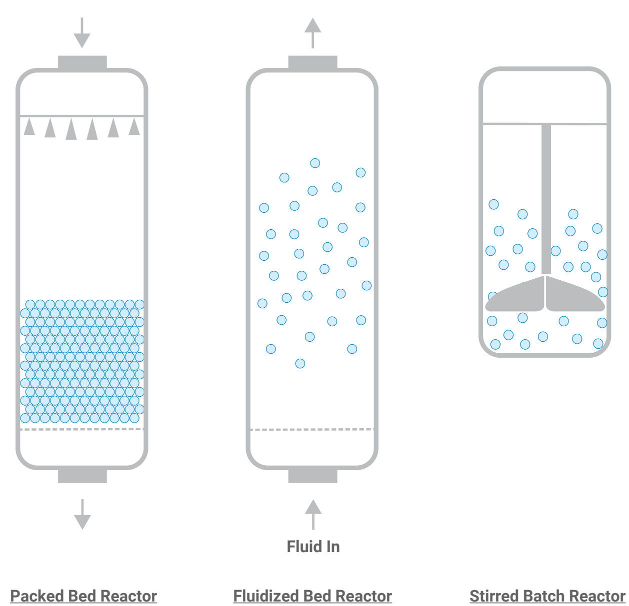
The most common enzymatic reactor for continuous operation is the packed-bed setup. This is a cylindrical column containing a fixed bed of catalyst particles (Figure 10). These particles should be larger in size to ensure pressure drop is kept within reasonable limits. Commonly operated in down-flow mode, the flow rate ranges used must provide a compromise between reasonable pressure drop, minimal diffusion layer and high conversion yield. Minimization of external mass-transfer resistances with enhanced flow rates can be considered, leading to the fluidized-bed reactor or expanded bed.

A fluidized-bed or expanded bed reactor is a variation of the packed-bed reactor, but operated in up-flow mode, where the biocatalyst particles are not in close contact which each other. This results in a lower pressure drop. The residence time allowed by the flow rates required for fluidization may, however, result in low conversion yields.

Bioconversions on smaller scales are typically carried out in stirred batch reactors. Shear stress induced by stirring can create a hazardous environment for immobilized biocatalysts as they are prone to abrasion. Mechanical stability of enzyme carriers such as the Lifetech™ ECR range is key for ensuring optimal performance in this type of configuration.

FIGURE 10

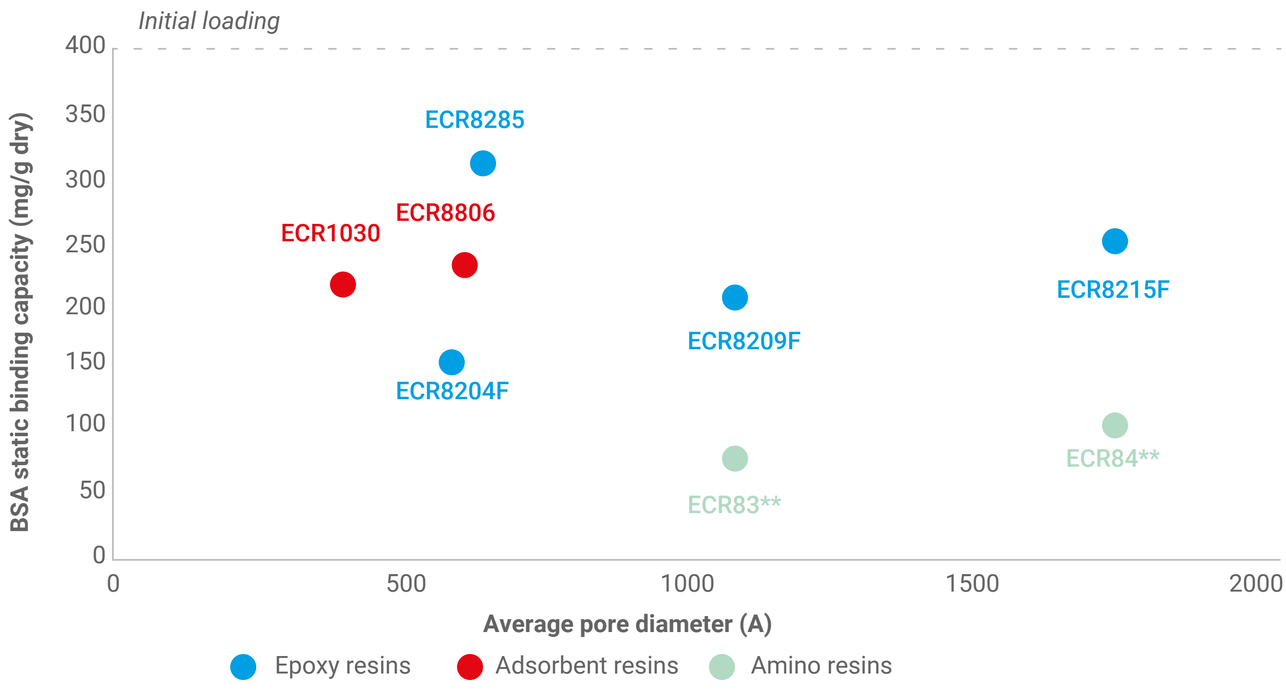
Common bioreactor configurations used with immobilized enzymes



Technical characteristics
Lifetech™ ECR resins | Static binding capacity

FIGURE 11

Typical protein binding capacity for Lifetech™ ECR resins is in the range of 50 - 300 mg protein per gram of dry resin.



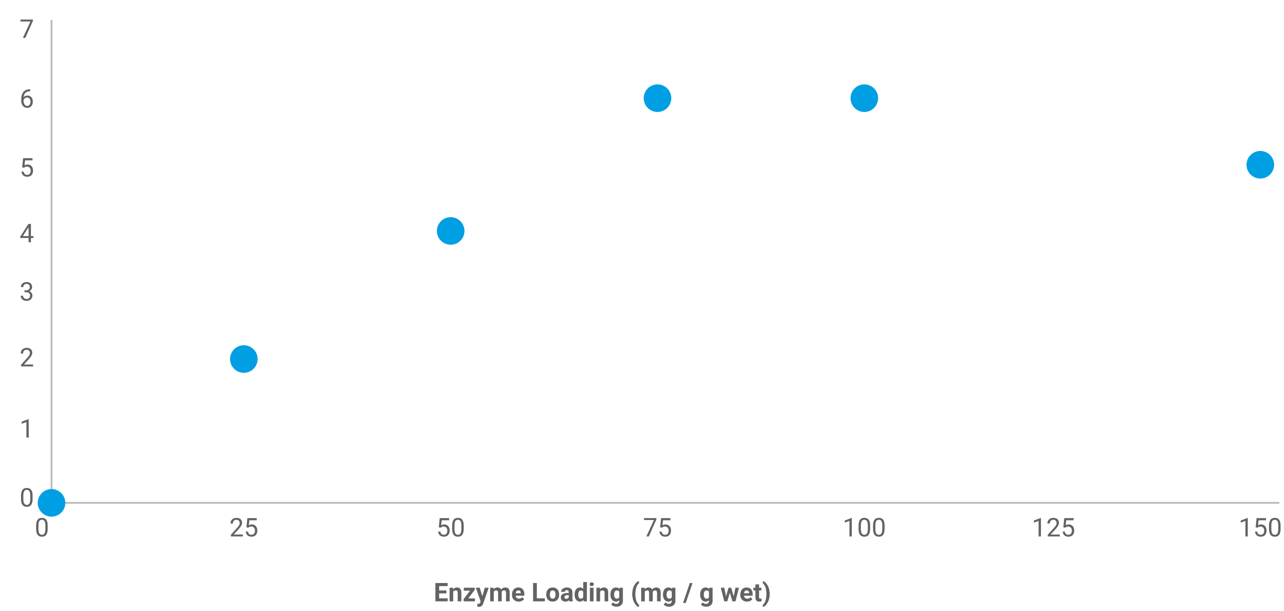
Lifetech™ ECR resins | Enzyme loading curves

When performing enzyme immobilization studies, we recommend running loading studies in order to obtain the optimal enzyme activity based on protein loading (Fig. 12).

Overloading the proteins does not necessarily result in higher enzyme activity due to steric hindrances.

FIGURE 12

An example of an enzyme loading study for Lifetech™ ECR8204F.



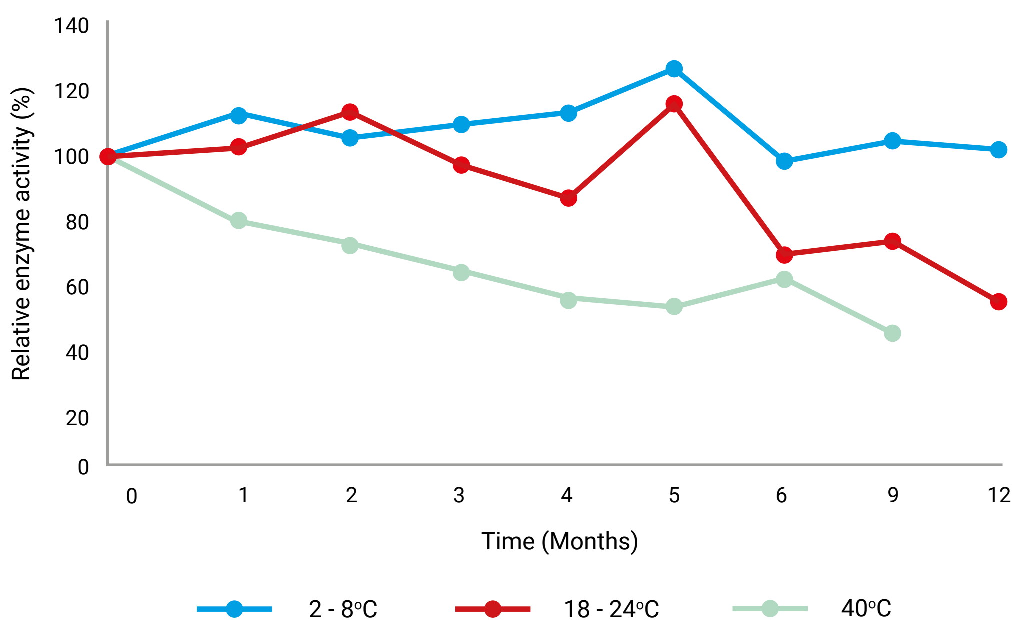
Lifetech™ ECR resins | Shelf life

ECR epoxy resins undergo typical degradation of epoxy rings which over time causes the opening of the epoxy rings to give diols. Internal studies have demonstrated that epoxy methacrylate resins are fully stable for a period of 6 months if stored in closed containers at 2-8°C. For shipping purposes ECR epoxy resins are stable out of the 2-8°C for short periods so there are no requirements of special refrigerated containers.

FIGURE 13

Stability of epoxy methacrylate ECR resins over time and at different temperatures

Figure 13 shows the stability profile of ECR8209F at refrigerated (2 - 8°C), room (18 - 24°C) and higher temperatures (40°C). Each were stored for a period of 12 months. Based on results the epoxy ECR are fully stable for a period of six months at 2-8°C. At each time point, Penicillin G amidase was immobilised on the ECR8209F, tested for activity and compared to the initial activity of fresh resin.



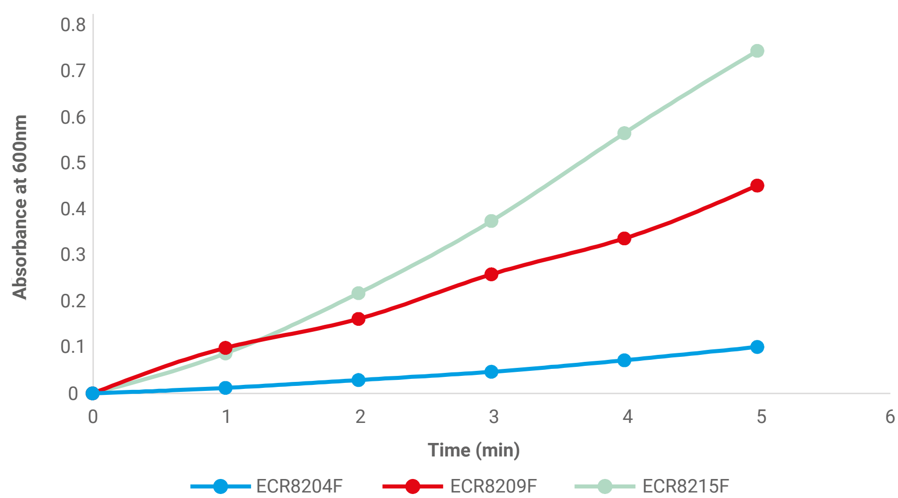
Lifetech^{TM} ECR resins | Mechanical stability

Lifetech™ ECR resins show outstanding mechanical stability when compared to other commercial resins. Mechanical stability is measured using an attrition test that determines the formation of fine particles. Increase in absorbance is related to the formation of particulates in the water due to breakage of the resin beads.

FIGURE 14

Mechanical stability of ECR8204F (300-600 Å pore diameter), ECR8209F (600-1200 Å pore diameter) and ECR8215F (1200-1800 Å pore diameter).

Figure 14 demonstrates how increased porosity results in lower mechanical stability. Therefore, highly porous resins are best suited for column applications rather than batch applications. The graph shows that mechanical stability for ECR8204F > ECR8209F > ECR8215F.

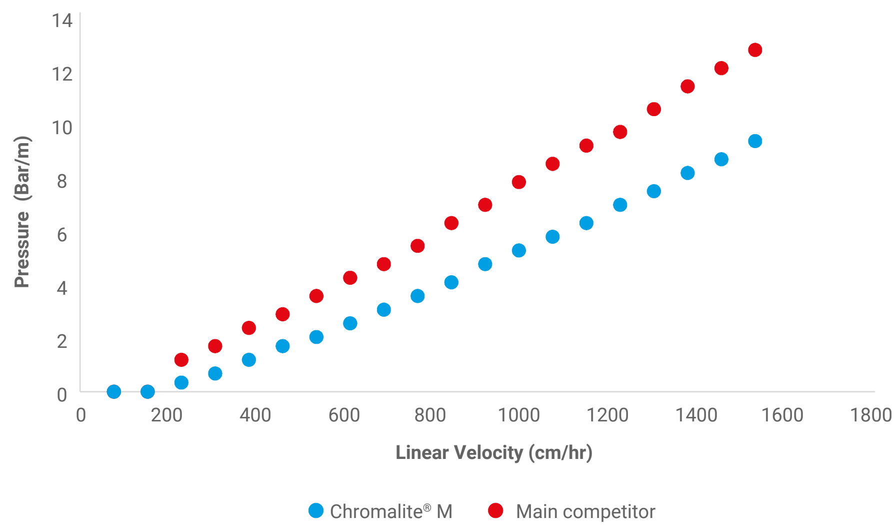


Chromalite® MIDA |
Pressure flow performance

FIGURE 15

Chromalite® MIDA pressure / flow performance

Figure 15 demonstrates that the resin backbone of Chromalite® MIDA is both robust and rigid, making it suitable for use in columns at very high flow rates.



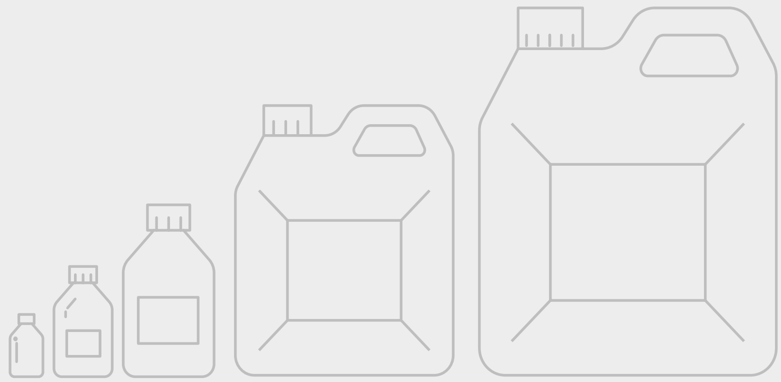
Packaging, particle sizes and ordering information

To place your order simply contact us via email or telephone and quote the product codes found on pages 33 and 34.

Packaging | Lifetech™ ECR resins

Standard packing is 50g, 250g, 1kg and 25kg. Resins are supplied in wet form and do not require a cleaning or washing pre-treatment before use. Typical bulk density for is 0.70 - 0.72 g/ml when supplied as wet.

Resin quantity	Container
50g resin	50g tub
250g resin	250g tub
1kg resin	1kg tub
5 - 25kg resin	5kg tub
25 - 100kg resin	60L keg
100 - 1000kg resin	200L keg
>1000kg resin	200L keg



Particle sizes | Lifetech™ ECR resins

Lifetech™ ECR resins are available in either F-grade (150 - 300 µm) or M-grade (300 - 710 µm) particle size ranges.

F-grade resins are optimal for research and pharmaceutical applications if batch volumes are limited and pressure drop during filtration is not significant. F-grade resins are also ideal for batch reactor configurations.

M-grade resins are ideal for large reaction volumes or viscous systems commonly found in fine chemical or food-based applications. They work best in packed bed or fluidized bed reactor configurations.

Your optimal product will depend on your processing goals. F grade products enable higher specific enzymatic activity to be obtained, whereas M grade products are easier to handle and reduce filtration time. If you need assistance with product selection, please contact lifesciences@purolite.com for expert technical support.

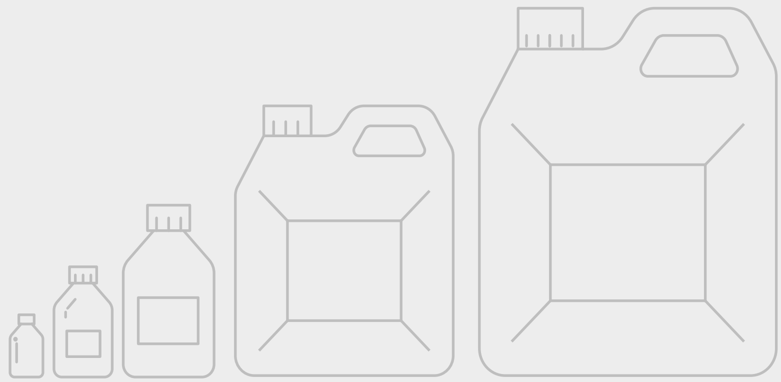
Ordering information | Lifetech™ ECR resins

Lifetech™ ECR Product	Product code
Lifetech™ ECR1090M	LS01105
Lifetech™ ECR8204F	LS01211
Lifetech™ ECR8204M	LS01215
Lifetech™ ECR8209F	LS01231
Lifetech™ ECR8209M	LS01235
Lifetech™ ECR8215F	LS01241
Lifetech™ ECR8215M	LS01245
Lifetech™ ECR8285	LS01269
Lifetech™ ECR8304F	LS01311
Lifetech™ ECR8304M	LS01315
Lifetech™ ECR8309F	LS01351
Lifetech™ ECR8309M	LS01355
Lifetech™ ECR8309M/PH4	LS01356
Lifetech™ ECR8315F	LS01361
Lifetech™ ECR8315M	LS01365
Lifetech™ ECR8404F	LS01371
Lifetech™ ECR8404M	LS01375
Lifetech™ ECR8409F	LS01381
Lifetech™ ECR8409M	LS01385
Lifetech™ ECR8415F	LS01391
Lifetech™ ECR8415M	LS01395
Lifetech™ ECR8806M	LS01415
Lifetech™ ECR1030M	LS01125
Lifetech™ ECRKIT1	LS01001-KIT

F grade particle size range is 150 - 300 μm
M grade particle size range is 300 - 710 μm
Typical bulk density for Lifetech™ ECR resins is 0.70 - 0.72 g/ml when supplied as wet.

Packaging | Chromalite® MIDA

Resin Volume	Container
25ml resin	75ml bottle
100ml resin	250ml bottle
500ml resin	1 litre bottle
1 litre resin	2.5 litre bottle
5 litres resin	10L Jerrycan
10 litres resin	20L Jerrycan
20 litres resin	130-litre Drum



Particle sizes | Chromalite® MIDA

Chromalite® MIDA is offered in the following particle size ranges:

- 40 - 90 µm (F-grade)
- 75 - 125 µm (M-grade)
- 100 - 300 µm (C-grade)

Ordering information | Chromalite® MIDA

Chromalite® M products	Product code
Chromalite® MIDA/F	LS04042
Chromalite® MIDA/M	LS04052
Chromalite® MIDA/C	LS04062
Chromalite® SpectraChrom KIT	LS04000-KIT

References

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

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