APPLICATION GUIDE

BIOCATALYSIS

PUROLITE® ECR Enzyme Immobilization Resins

Purolite®

APPLICATION GUIDE

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 the 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 which are often an essential prerequisite for establishing an enzyme-catalyzed process in the first place.

The applications using immobilized enzymes offer high degree of flexibility, being suitable for continuous processes using fixed or expanded-bed reactors or in batch 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.
There are critical parameters in the preparation of immobilized biocatalysts, such as the immobilization yield, mass transfer limitations and operational stability, which have a high impact on the efficacy of the system and are tightly controlled. Operative binding forces vary between weak multiple adsorptive interactions and single attachments through strong covalent binding. Which of the methods is the most appropriate is usually determined by the application parameters.

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

Table 1 – Examples of immobilized enzymes in industrial applications

Enzyme	Substrate	Product	Amount [ton/y]	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 β-decarboxylase	Aspartic acid	L-Alanine	120	*Chemicals*
Cephalosporin amidase	Glutaryl-7-ACA	7-ACA	unknown	*Pharmaceutical*
Lactase (galactosidase)	Lactose	Lactose free milk	unknown	*Dairy*

PARAMETERS AFFECTING THE PERFORMANCE OF IMMOBILIZED ENZYMES

The properties of immobilized enzyme preparations are governed 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).

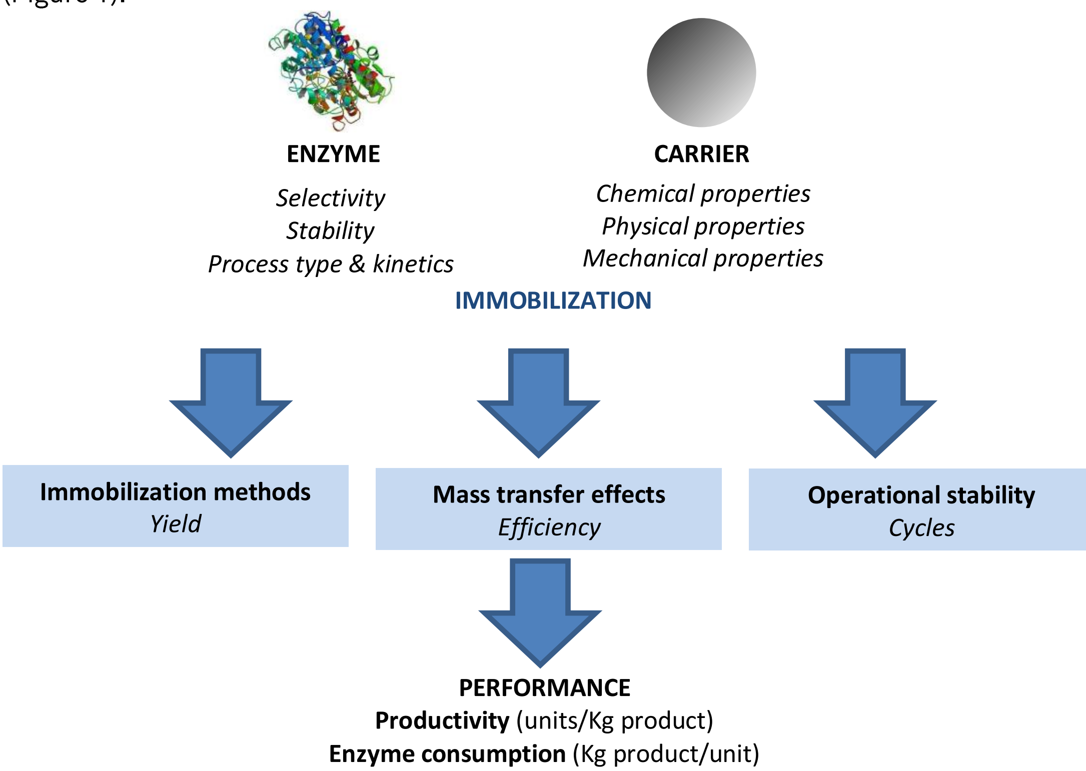


Figure 1 – Parameters affecting the performance of immobilized enzymes

As far as 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 may be further reduced as a result of 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 we replace “enzyme units” by “enzyme costs” we obtain the essential product related costs, for example, in US$ per kg of product.

PROPERTIES OF CARRIERS FOR ENZYME IMMOBILIZATION

The characteristics of the carrier have a strong influence on the performance of an immobilized enzyme. The following properties should be well selected and balanced for a specific biotransformation (see Table 2 and Figure 2 below):

· **Functional groups:** The type of activation, presence, distribution and density of functional groups determines the activity yields of an immobilization reaction, the stability and operational stability of the carrier-fixed enzyme. Purolite® ECR resins are offered in a variety of functional groups and customizations are available.

· **Porosity and Surface area:** In most cases, a large surface area (>100 m2/g) and high porosity are desirable so that enzyme and substrate can be easily penetrated. A pore size of >300 Å is usually adequate to make the internal surface accessible for immobilization of most enzymes. Purolite® ECR resins are offered with different degree of porosity and surface area, usually higher than 400Å and >40 m2/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. Purolite® ECR resins are made of styrene, acrylate or styrene/acrylate 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. Purolite® ECR resins are rigid and spherical beads and can be used for applications in batch or bed 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. Purolite® ECR resins are designed to be mechanically stable, allowing their use in repeated cycles.

· **Form and size of support:** The particle size will have an influence on filtration times from stirred tank reactors in repeated batch mode. Furthermore, this factor is important for the performance in column reactors regarding back pressure and flow rates, which of course are correlated. For this purpose a size of spherical particles in the range of 150 - 300 μm is preferred. Purolite® ECR resins are offered in two particle sizes, 150 - 300 μm and 300 -700 μm. These two ranges cover almost all types of applications from lab to the industrial reactor.

· **Resistance to microbial attack:** During long term usage the support has to be stable against microbial degradation. Purolite® 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. Purolite® ECR resins for adsorption can be regenerated and reused for further enzyme immobilization.

· **Safety and regulations:** Purolite® 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.[2]

1. Epoxy resins undergo typical degradation of epoxy resins. Purolite® ECR resins are supplied with indication of time of usage before expiring.

2. Purolite ECR1811 Octadecyl styrene is not food grade.

PUROLITE ECR RESINS FOR ENZYME IMMOBILIZATION

Figure 2 – The different chemistries that are available from Purolite LifeTech™ for enzyme immobilization.

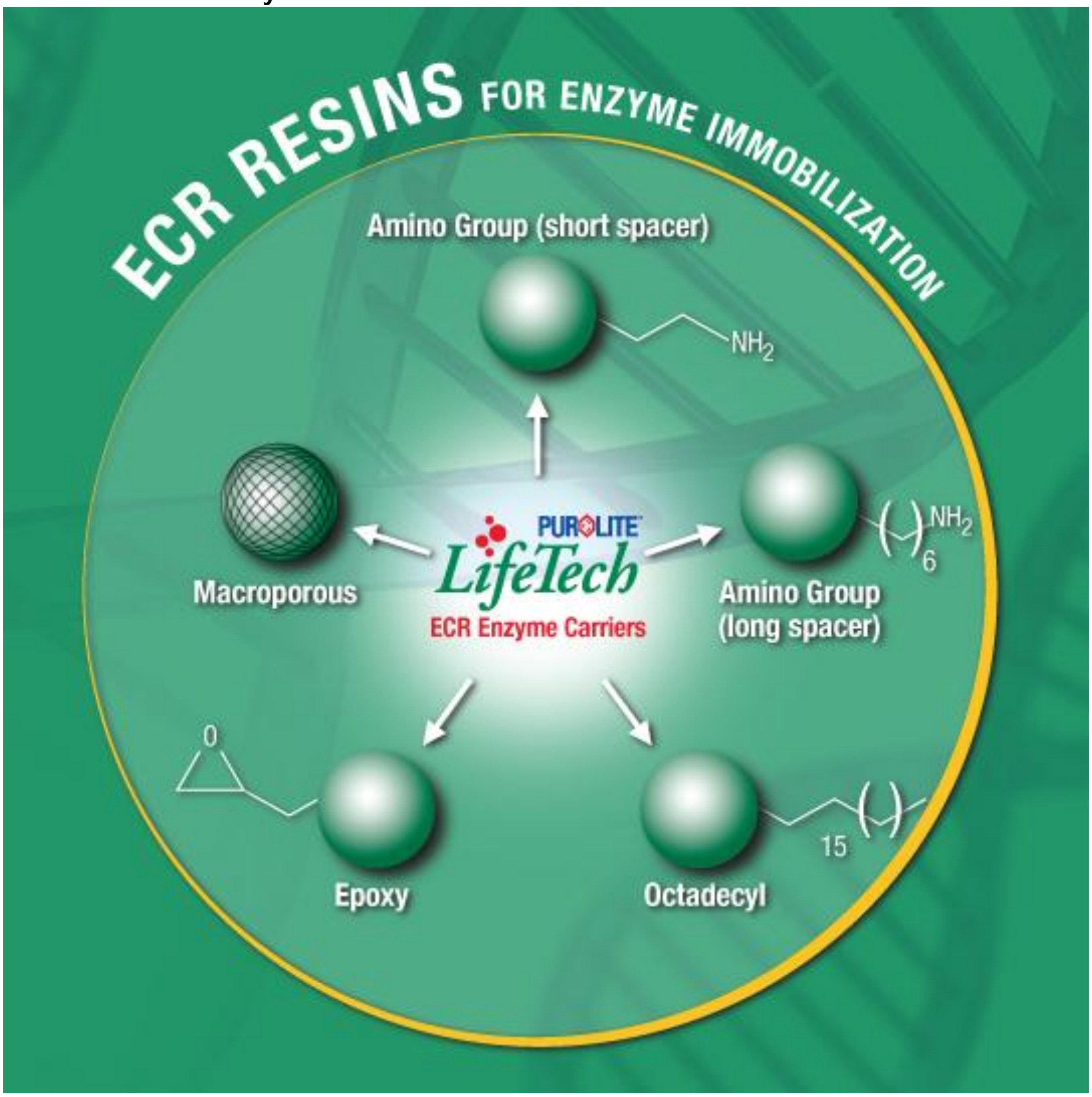


Table 2 – Immobilization methods, applications and technical features for Purolite ECR resins.

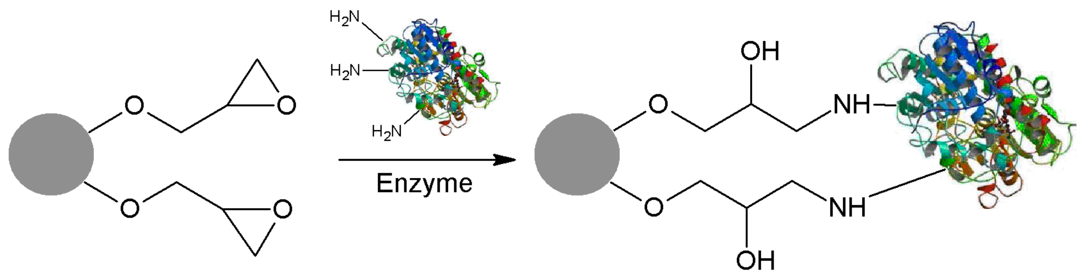
Product	Functional Group	Immobilization	Application	Surface Area (m^2/g)[a]	Pore Diameter (Å)[b]	Water Content (%)[c]
ECR8205 Epoxy acrylate	Epoxy	Covalent	H2O/non-H2O	> 80	450 - 600	52 - 57
ECR8214 Epoxy acrylate	Epoxy	Covalent	H2O/non-H2O	> 60	1200 - 1800	60 - 66
ECR4204 Epoxy acrylic/styrene	Epoxy	Covalent	H2O/non-H2O	> 140	275 - 450	41 - 46
ECR8310 Amino C2 acrylate	NH2 (short spacer)	Covalent	H2O/non-H2O	> 70	850 - 1200	58 - 62
ECR8319 Amino C2 acrylate	NH2 (short spacer)	Covalent	H2O/non-H2O	> 50	1600 - 2000	62 - 66
ECR8405 Amino C6 acrylate	NH2 (long spacer)	Covalent	H2O/non-H2O	> 40	450 - 850	50 - 55
ECR8417 Amino C6 acrylate	NH2 (long spacer)	Covalent	H2O/non-H2O	> 50	1600 - 2200	61 - 65
ECR8804 Octadecyl acrylate	Octadecyl	Adsorption	non-H2O	N/A	350 - 450	45 - 50
ECR8806 Octadecyl acrylate	Octadecyl	Adsorption	non-H2O	> 80	500 - 700	58 - 63
ECR1811 Octadecyl styrene	Octadecyl	Adsorption	non-H2O	> 200	1000 - 1200	59 - 63
ECR1090 Macroporous styrene	None	Adsorption	non-H2O	> 750	900 - 1100	67 - 73
ECR1091 Macroporous styrene	None	Adsorption	non-H2O	> 450	950 - 1200	62 - 68

[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

Epoxy 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 ECR8205 Epoxy acrylate and **Purolite ECR8214** Epoxy acrylate are epoxy-activated resins produced via very intense crosslinking in the presence of a porogenic agent that allows the control of porosity. Two standard porosity grades are available (450 - 600Å and 1200 - 1800Å respectively, see Table 2). These carriers are stable during storage and easy to handle before, after and during the immobilization procedures. **Purolite ECR8205** and **Purolite ECR8214** are designed to form very stable covalent linkages with different protein groups (amino, thiol, phenolic) under very mild experimental conditions of pH and temperature (Figure 3). The resins are mechanically very stable and the final immobilized biocatalysts can be used in either stirred tank or bed reactor. Performance of **Purolite ECR8214** Epoxy acrylate and **Purolite ECR8205** Epoxy acrylate in the immobilization of enzymes are excellent compared to other commercial products.



Purolite ECR Epoxy resin **Covalently immobilized enzyme**

Figure 3 – Immobilization of enzymes on epoxy carriers

Purolite ECR4204 Epoxy acrylic/styrene is an epoxy activated carrier with a matrix of styrene and acrylate. This combination is optimal in the immobilization of enzymes that work in biphasic systems or aqueous 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.

Amino 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.

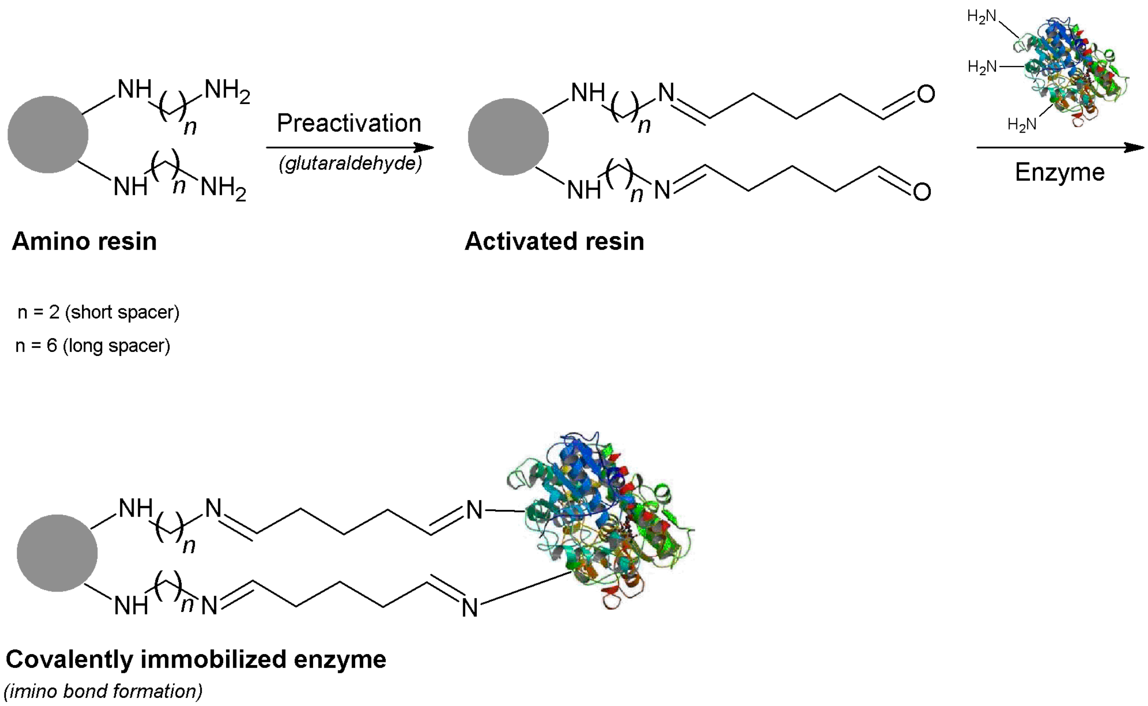


Figure 4 – Immobilization of enzymes on amino carriers

Purolite LifeTech™ offers different amino resins, with short and long spacer (see Table 2). **Purolite ECR8310** Amino C2 acrylate and **Purolite ECR8319** Amino C2 acrylate are amino resins with short ethylene spacer and two different porosities: 850 - 1200Å and 1600 - 2000Å respectively.

Purolite ECR8405 Amino C6 acrylate and **Purolite ECR8417** Amino C6 acrylate are amino resins with a long hexamethylene spacer and two different porosities: 450 - 850Å and 1600 - 2200Å respectively.

Resins for enzyme adsorption

This method for the immobilization of enzymes is based on the physical adsorption of enzyme protein 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 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.

Purolite® ECR resins include acrylic or styrene resins, with different degrees of hydrophobicity and porosity. These adsorbents are optimal for the immobilization of lipases.

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).

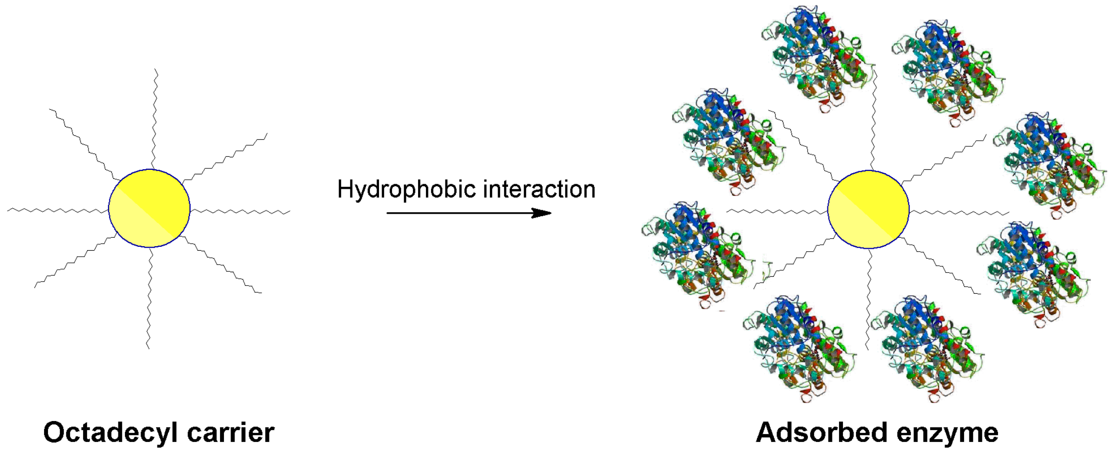


Figure 5 – Immobilization of enzymes on octadecyl-activated carriers

Purolite ECR8804/ECR8806 Octadecyl acrylate are octadecyl (C18) activated resins produced via a very intense crosslinking process in the presence of a porogenic agent that allows the control of porosity (350 - 450Å and 500 - 700Å respectively). Because of their optimized amount of octadecyl groups on the matrix, the performance of **Purolite ECR8804** and **ECR8806** Octadecyl acrylate in the immobilization of enzymes are 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 bed reactors.

Purolite ECR1811 Octadecyl styrene is and octadecyl (C18) activated resin produced via a very intense crosslinking in the presence of a porogenic agent that allows the calibration of porosity. This products has a pore diameter in the range of 1000 - 1200Å. The resin is more hydrophobic than **Purolite ECR8804** and **ECR8806** Octadecyl acrylate. **Purolite ECR1811** Octadecyl styrene is mechanically very stable and the final immobilized biocatalyst is suitable for use in both stirred tank and bed reactors.

Purolite ECR1090 and **ECR1091** Macroporous styrene are styrene resins with high degree of hydrophobicity. These resins are optimal for the immobilization of lipases, stable during storage and can easily be handled before and after immobilization procedures (Figure 6).

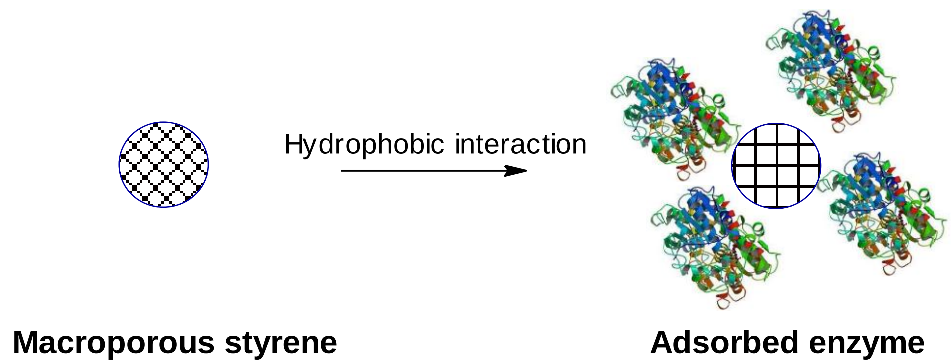


Figure 6 – Immobilization of enzymes on macroporous styrene carriers

Purolite ECR1090 and **ECR1091** Macroporous styrene are produced via a very intense 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. **Purolite ECR1090** and **ECR1091** Macroporous styrene are mechanically very stable and the final immobilized biocatalysts can be used in both stirred tank and bed reactor.

Particle size, packaging and available kits

All Purolite ECR resins are available with the following mean particle size:
F Grade: 150 - 300 µm (100 - 50 mesh)
M Grade: 300 - 700 µm (50 - 25 mesh)

The standard packing is 50g, 500g, 1kg and 5kg.

Resins are supplied in wet form (Table 2) and do not require any treatment before use.

Purolite ECRKIT1 enzyme carrier kit is a kit designed for screening purposes with sample sizes of 50g or 500g (Table 3).

Table 3 – Content of Purolite ECRKIT1 enzyme carrier kit

Kit Content	Functional Group	Immobilization
ECR8214F Epoxy acrylate	Epoxy	Covalent
ECR4204F Epoxy acrylic/styrene	Epoxy	Covalent
ECR8405F Amino C6 acrylate	NH2 (long spacer)	Covalent
ECR8405F Amino C6 acrylate	NH2 (long spacer)	Covalent
ECR8804F Octadecyl acrylate	Octadecyl	Adsorption
ECR1090F Macroporous styrene	None	Adsorption
Aspartase β-decarboxylase	Aspartic acid	L-Alanine
Cephalosporin amidase	Glutaryl-7-ACA	7-ACA
Lactase (galactosidase)	Lactose	Lactose free milk

Americas
150 Monument Road
Bala Cynwyd, PA
19004
T +1 800.343.1500
T +1 610.668.9090
F +1 484.384.2751
Americas@purolite.com

Europe
Llantrisant Business Park
Llantrisant
Wales, UK
CF72 8LF
T +44 1443 229334
F +44 1443 227073
Europe@purolite.com

Asia Pacific
Room 707, C Section
Huanglong Century Plaza
No.3 Hangda Road
Hangzhou, Zhejiang, China 310007
T +86 571 876 31382
F +86 571 876 31385
AsiaPacific@purolite.com

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