APPLICATION GUIDE

Lifetech™ ECR Enzyme Immobilization Procedures

Lifetech ECR resins are robust enzyme carriers designed for research and industrial applications. This guide provides step-by-step immobilization protocols for Lifetech ECR epoxy resins, Lifetech ECR amino resins, Lifetech ECR adsorbents and Lifetech ECR enzyme carriers for ionic immobilization. The guide also presents helpful information on selecting, working with and testing immobilized enzymes.

Purolite® Life Sciences

Inside this Application Guide you will find an overview of Purolite® Lifetech™ ECR Enzyme Immobilization Resins Procedures that enable easy separation of the enzyme from the product as well as reuseof 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.

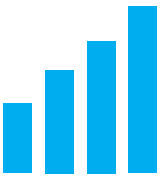
Responding to the needs of our customers, Purolite has built the largest technical sales force in the industry, the widest variety of products and five strategically located Research and Development groups. Our ISO 9001 certified manufacturing facilities in the U.S.A, UK, Romania and China combined with 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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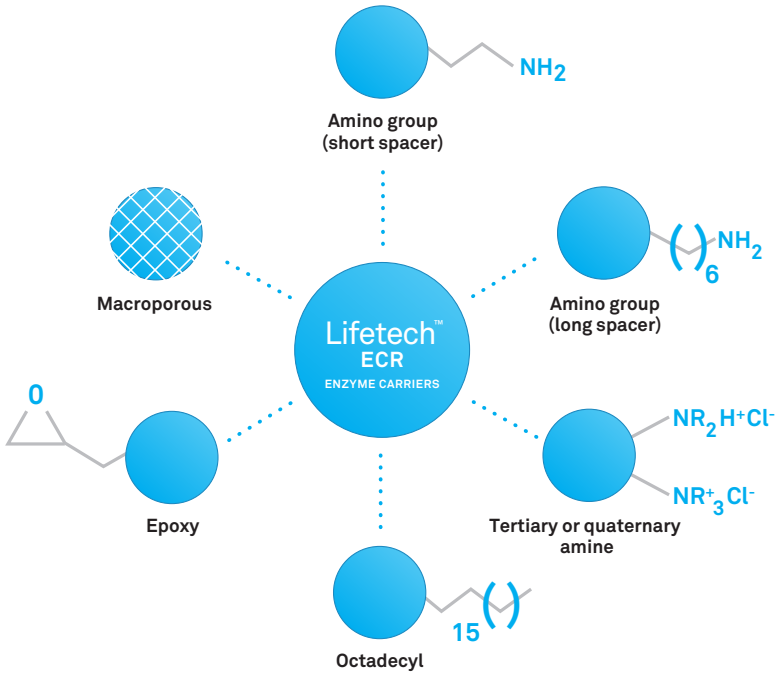
Figure 1 – Lifetech ECR resins for enzyme immobilization



Lifetech ECR enzyme carriers

Lifetech ECR resins are robust enzyme carriers designed for research and industrial applications. The wide range of functional groups (Figure 1), porosities, matrices and particle size provide the largest library for enzyme immobilization in the industry.

Immobilized enzymes are applied in a variety of processes and provide several advantages such as:

- Multiple reuses of the biocatalyst
- Easy separation of the enzyme from the product
- Flexibility in reactor design
- Ability to regenerate the carrier
- Possibility to operate in both aqueous organic solvents
- Possibility to operate in a continuous mode using column rectors

Lifetech ECR resins are designed for covalent, adsorptive and ionic immobilizations.

Covalent immobilization is a method for immobilizing enzymes based on the formation of chemically stable covalent linkages with different functional groups of the enzymes (amino, thiol, phenolic) with the epoxy groups in the carrier, under very mild reaction conditions.

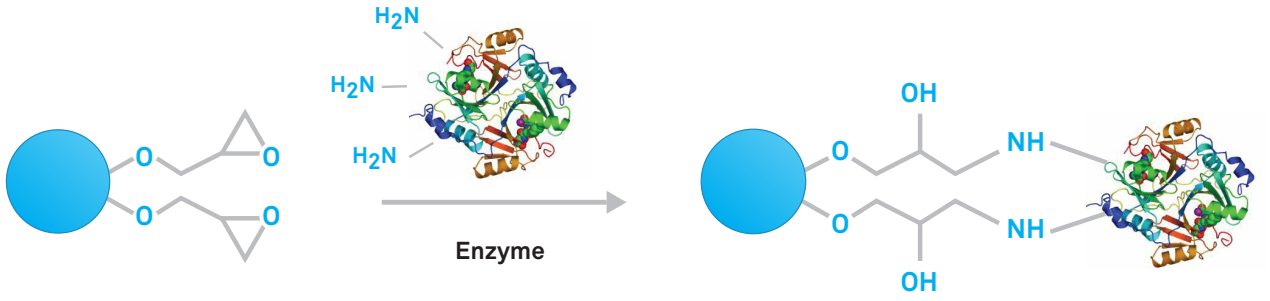
Adsorption is a physical process that exploits hydrophobic interaction between the hydrophobic surface of enzyme (due to the presence of areas with aminoacids like Phe, Trp, Leu as an example) and the carrier (polymer). Physical interactions can be very robust and allow the biocatalyst to be used for many cycles, especially in water free-media.

Ionic immobilization is a physical process that can combine both ionic interaction between charged proteins and oppositely charged carriers and the adsorption onto hydrophobic carrier. The immobilized enzyme is stable and can be used in many industrial processes. This mechanism of immobilization also allows regeneration of the carrier after enzyme exhaustion.

Covalent immobilization using epoxy resins

Epoxy-activated resins are ideal matrices to perform easy immobilization of enzymes since they allow multipoint covalent binding between enzyme and resin (Figure 2). Immobilization on Lifetech ECR epoxy resins is more efficient using high ionic strength buffers.

Figure 2 – Immobilization using Lifetech ECR epoxy resins



Purolite ECR Epoxy resin **Covalently immobilized enzyme**

Lifetech ECR Epoxy resins designed for covalent immobilization of enzymes include four products as described in Table 1.

Table 1 – Lifetech ECR epoxy resins and their properties

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

† Available as F grade (150 - 300 µm), M grade (300 - 710 µm) and full grade (100 - 710 µm).
†† ECR8285 is designed – thanks to the presence of butyl groups – for immobilization of hydrophobic enzymes, showing remarkable results with lipase. For more information, see the Purolite Lifetech ECR Enzyme Immobilization Resins brochure.

Immobilization protocol using Lifetech ECR epoxy resins

Reagents

- Lifetech ECR Epoxy resins.
- Native enzyme in liquid or solid form (i.e. lyophilized).
- Immobilization buffer: use a buffer that is compatible with enzyme activity and stability. Immobilization on epoxy resins is more efficient when using highly concentrated buffers (about 1 M or higher). However, buffers at concentrations > 1 M are difficult to be applied in industrial conditions due to limited solubility of salts.
- Washing buffer for desorption of non-covalently bound enzyme from the support. (Buffer with strength of about 0.01 - 0.02 M or deionized water).

Procedure

1. **Resin equilibration**
   - Wash the resin with immobilization buffer and filter. A resin/buffer ratio of 1/1 (w/v) is preferable. Repeat the process for 2 - 4 times.

2. **Preparation of the enzyme solution**
   - Dissolve the native enzyme (liquid or solid) in immobilization buffer.
   - Consider a protein loading of 50 - 100 mg protein per gram of wet resin. Protein concentration can be determined by using standard protein content assays.
   - Dissolve the enzyme in a sufficient amount of buffer to obtain a ratio resin/buffer of 1/4 (w/v).
   - Optimization of this ratio can be pursued in further trials (range can vary from 1/1 - 1/4).

3. **Immobilization**
   - Transfer the immobilization buffer containing the enzyme into the immobilization vessel. Add the Lifetech ECR Epoxy resin.
   - Mix the slurry gently (at 70 - 80 rpm) for 18 h. Stop after 18 h and leave without mixing for another 20 h. **Note: avoid using magnetic stirring during enzyme immobilization as this can damage beads.**
   - Immobilization can be performed at temperatures of 20°C - 30°C, depending on enzyme stability.
   - Do not perform immobilizations at high temperatures as this can cause degradation of the epoxy rings (hydrolysis) and facilitate microbial growth.

4. **Filtration and washing**
   - Filter the liquid phase and collect it.
   - Determine the protein content in the liquid and evaluate the immobilization yield.
   - Wash the resin with washing buffer. Repeat process for 2 - 4 times, under gentle stirring or in column wash. An additional washing step using a 0.5 M NaCl containing buffer for complete desorption of non-covalently bound proteins is recommended.
   - Remove the excess of water by filtration.

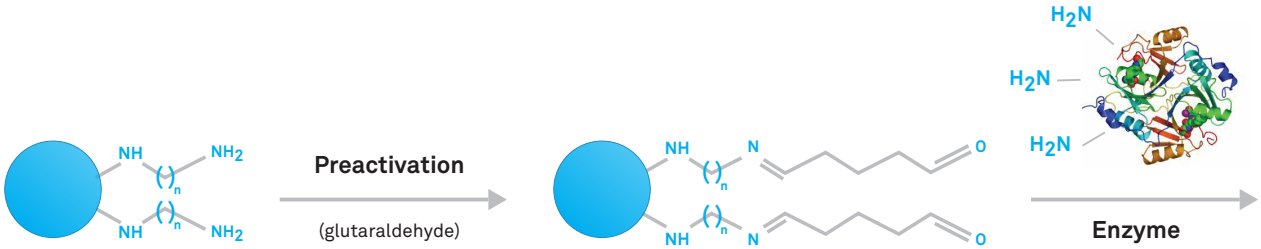
5. **Characterization**
   - Characterize the immobilized enzyme in terms of moisture content and specific activity.

6. **Storage**
   - Transfer the immobilized enzyme into a suitable container and keep refrigerated 2°C - 8°C. **Note: avoid freezing the immobilized enzyme since this may damage the beads.**

Covalent immobilization using amino resins

Another procedure for covalent immobilization of enzymes is based on the use of amino resins. Amino resins can be pre-activated with glutaraldehyde and then used in for covalent immobilization of enzymes (Figure 3). Reaction of an aldehyde group with an amino group of the enzymes is fast and forms a *Schiff base* (imine), resulting in a stable multipoint covalent binding between enzyme and carrier. An even more stable linkage can be achieved by reduction of the imine double bonds with borohydrides.

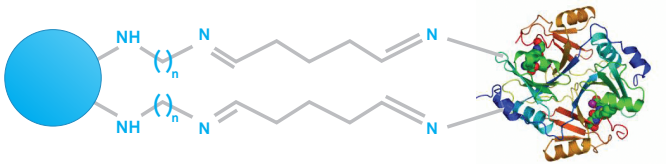
Figure 3 – Covalent immobilization using Lifetech ECR amino resins



Amino resin

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

Activated resin



Covalently immobilized enzyme
(imino bond formation)

Lifetech ECR amino resins include the products that are described in Table 2. These products are specially designed for covalent immobilization of enzymes.

Table 2 – Lifetech ECR amino resins and their properties

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

†Available as F grade (150 - 300 µm), M grade (300 - 710 µm) and full grade (100 - 710 µm).

Immobilization protocol using Lifetech ECR amino resins

Reagents

- Lifetech ECR amino resins.
- Native enzyme in liquid or solid form.
- Immobilization buffer: use a buffer that is compatible with enzyme activity and stability. Immobilization on amino resins is efficient when using buffers of low concentration (0.01 - 0.05 M).
- Glutaraldehyde 25% (w/v).
- Washing buffer for desorption of non-covalently bound enzyme from the support (Buffer with strength of about 0.01 - 0.02 M or deionized water).

Procedure

1. **Resin equilibration**
   - Wash the resin with immobilization buffer and filter. A resin/buffer ratio of 1/1 (w/v) is preferable.

2. **Preparation of 2% glutaraldehyde buffer**
   - Starting from a solution of 25% (w/v) glutaraldehyde, prepare a 2% glutaraldehyde (v/v) solution using the immobilization buffer.

3. **Pre-activation of the amino resin**
   - Add the 2% glutaraldehyde buffer prepared in Step 2 to the resin. The optimal volume of 2% glutaraldehyde buffer should be in the range of resin/buffer ratio of 1/4 (w/v).
   - Leave the slurry to mix for 60 min at 20°C - 25°C.
   - Filter and wash the beads with immobilization buffer using a resin/buffer ratio of 1/4 (w/v).
   - Avoid storing pre-activated resin for a period longer than 48 h.
     Note: A change in color of the beads (orange-brown) may occur and is normal.
   - Beads are then ready for the immobilization step.

4. **Prepare enzyme solution**
   - Dissolve the native enzyme (liquid or solid) in immobilization buffer.
   - Consider a protein loading of 50 - 100 mg protein per gram of wet resin. Protein concentration can be determined by using standard protein content assays.
   - Dissolve the enzyme in buffer to obtain a ratio resin/buffer of 1/4 (w/v).
   - Optimization of this ratio can be pursued in further trials (range can vary from 1/1 - 1/4).

5. **Immobilization**
   - Transfer the immobilization buffer into the immobilization vessel and add the Lifetech ECR pre-activated amino resin as prepared in Step 3.
   - Mix the slurry gently for 18 h at 70 - 80 rpm.
     Note: avoid using magnetic stirring during enzyme immobilization as this can damage beads.
   - The immobilization can be performed at 20°C - 30°C accordingly to enzyme stability.
   - Do not perform immobilizations at high temperatures since this might cause side reactions of the aldehyde groups on the resin formed during Step 3.

6. **Filtration and washing**
   - Filter the liquid phase, collect it and determine the protein content in the liquid for immobilization yield.
   - Wash the resin with immobilization buffer. An additional washing step using a 0.5 M NaCl containing buffer for complete desorption of non-covalently bound proteins is recommended.

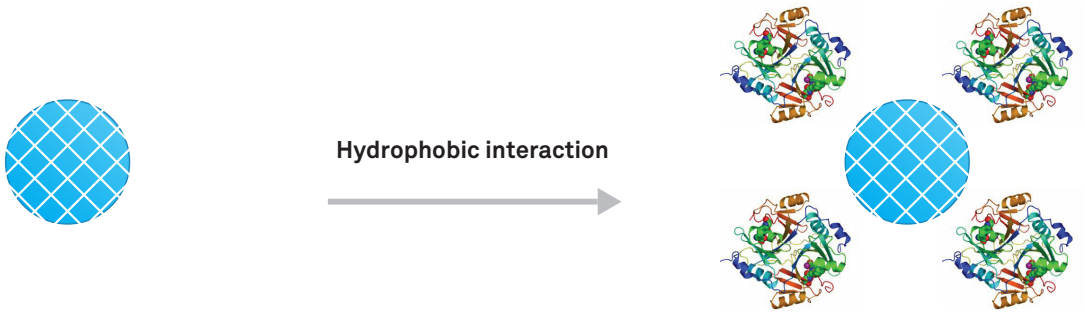
7. **Characterization**
   - Characterize the immobilized enzyme in terms of moisture content and specific activity.

8. **Storage**
   - Transfer the immobilized enzyme into a suitable container and keep refrigerated 2°C - 8°C.
     Note: avoid freezing the immobilized enzyme since this may damage the beads.

Immobilization by adsorption

This method for the immobilization of enzymes is based on the physical adsorption of enzymes on the surface of water-insoluble carriers (Figure 4). The method is very gentle and causes little or no conformational changes of the enzyme or denaturation of its active site. This method is particularly suitable for applications in hydrophobic media, such as organic solvents and oils. A major advantage of adsorption as a general method of immobilizing enzymes is that usually no additional reagents are required.

Figure 4 – Immobilization on Lifetech ECR macroporous styrene resins or octadecyl resins



Lifetech ECR resins for adsorption include several products that are described in Table 3. These products are designed for non-covalent immobilization of enzymes.

Table 3 – Lifetech ECR octadecyl and macroporous resins and their properties

LIFETECH PRODUCT†	TYPE	FUNCTIONAL GROUP	SURFACE AREA (m^2/g)	PORE DIAMETER (Å)	MOISTURE (%)
ECR8804	Octadecyl methacrylate	Octadecyl	N/A	350 - 450	45 - 50
ECR8806	Octadecyl methacrylate	Octadecyl	> 80	500 - 700	58 - 63
ECR1090	Macroporous styrene	None	> 750	900 - 1100	67 - 73
ECR1091	Macroporous styrene	None	> 450	950 - 1200	62 - 68
ECR1061	DVB/methacrylate	None	> 400	600 - 750	60 - 70
ECR1030	DVB/methacrylate	None	> 90	200 - 300	57 - 63

† Available as F grade (150 - 300 µm), M grade (300 - 710 µm) and full grade (150-710 µm). ECR1030 and ECR1061 are available only in M grade.

Immobilization protocol using Lifetech ECR adsorbents

Reagents

- Lifetech ECR Macroporous or Octadecyl resins.
- Native enzyme in liquid or solid form.
- Immobilization buffer: use a buffer that is compatible with enzyme activity and stability. Immobilization can be performed using low concentration buffers (about 0.01 - 0.05 M).

Procedure

1. **Resin equilibration**
   - Wash the resin with immobilization buffer and filter. A resin/buffer ratio of 1/1 (w/v) is preferable.

2. **Preparation of the enzyme solution**
   - Dissolve the native enzyme (liquid or solid) in immobilization buffer.
   - Consider a protein loading of 50 - 100 mg protein per gram of wet resin. Protein concentration can be determined by using standard protein content assays.
   - Dissolve the enzyme in buffer to obtain a 1/4 (w/v) resin/buffer ratio.

3. **Immobilization**
   - Transfer the immobilization buffer into the immobilization vessel and add the Lifetech ECR resins.
   - Mix the slurry for 24 h at 70 - 80 rpm.
     Note: avoid using magnetic stirring during enzyme immobilization as this can damage beads.
   - The immobilization can be performed at temperatures above 20°C depending on enzyme stability.

4. **Filtration and washing**
   - Filter the liquid phase and collect it.
   - Determine the protein content in the liquid and evaluate the immobilization yield.
   - Wash the resin once with washing buffer (ratio resin/buffer of 1/1 (w/v).

5. **Characterization**
   - Characterize the immobilized enzyme in terms of moisture content and specific activity.

6. **Storage**
   - Transfer the immobilized enzyme into a suitable container and keep refrigerated 2°C - 8°C.
     Note: avoid freezing the immobilized enzyme since this may damage the beads.

7. **Drying**
   - Adsorbed immobilized enzymes may need to be dried prior to use. See “Additional information,” on page 12.

Ionic immobilization using tertiary or quaternary amine resins

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

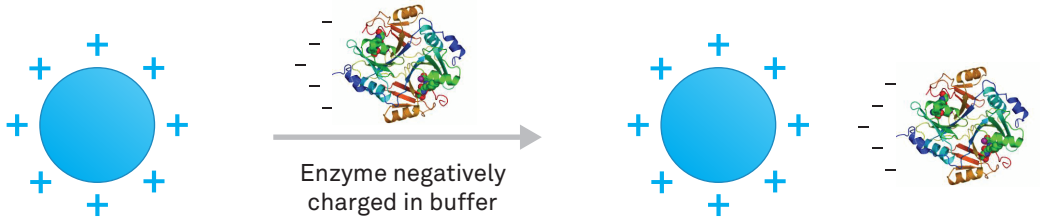
Ionic immobilization is the simplest of all the immobilization techniques that does not grossly alter the activity of the bound enzyme. In case of enzymes immobilized through ionic interactions, adsorption and desorption of the enzyme depends on the basicity of the ion exchanger. The dynamic equilibrium 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 is used successfully in many industrial food processes.

Lifetech ECR ionic resins have been successfully applied in immobilization of (specific protocols are available on request):

- Invertase
- Glucosyltransferase
- Glucoamylase
- Galactosidase
- Lipase RM
- Lipase TL

In addition to the products listed in Table 4, all Lifetech ECR amino resins (see Table 2) can also be used for immobilization by ionic interaction using same protocol described here. Lifetech ECR8309, as an example, shows excellent industrial performance for the immobilization of glucoamylase.

Figure 5 – Ionic enzyme immobilization using Lifetech ECR enzyme carriers



Charged resins

Immobilized enzyme through ionic interaction

Table 4 – Lifetech ECR enzyme carriers for ionic enzyme immobilization

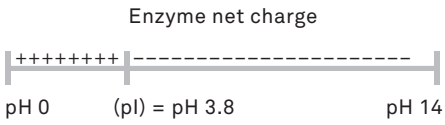
LIFETECH PRODUCT†	TYPE	FUNCTIONAL GROUP	IMMOBILIZATION	MOISTURE RETENTION (%)	TOTAL CAPACITY (eq/l)
ECR1504	Styrene tertiary amine	$-NR_2$	Ionic	53 - 62	1.3
ECR1508	Styrene tertiary amine	$-NR_2$	Ionic	51 - 58	1.5
ECR1604	Styrene quaternary amine	$-NR_3^+Cl^-$	Ionic	53 - 58	1.15
ECR1640	Styrene quaternary amine	$-NR_3^+Cl^-$	Ionic	66 - 72	0.85

† 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.

Figure 6 illustrates the selection of appropriate operative pH buffer for an enzyme with a pI of 3.8. If the optimal pH of the enzyme or its operation pH is higher than its pI, then the enzyme will be negatively charged. In this case, a tertiary or quaternary amine resin with positive charges will be optimal for ionic immobilization.

Figure 6 – Selecting the appropriate operative pH for an enzyme with isoelectric point of 3.8

Weak or strong base anion resin



Example isolelectric point for an enzyme (pI = 3.8)

Immobilization protocol using Lifetech ECR resins for ionic immobilization

Reagents

- Lifetech ECR base anion resins.
- Native enzyme in liquid or solid form.
- Immobilization buffer: use a buffer that is compatible with enzyme activity and stability. Consider the isoelectric point (pI) of the enzyme and operative pH for buffer selection. Isoelectric point value can be obtained in enzyme databases such as Brenda, www.brenda-enzymes.org.

Procedure

1. **Resin equilibration**
   - Equilibrate the resin in deionized water with pH adjusted to a value above the pI of the enzyme by 1M HCl using a pH titrator (on laboratory scale) or using 2BV of 4 - 6% HCl (plant).
   - Filter the resin after pH adjustment and rinse with deionized water.

2. **Preparation of the enzyme solution**
   - Dissolve the native enzyme (liquid or solid) in immobilization buffer.
   - Consider a protein loading of 50 - 200 mg protein per gram of wet resin. Protein concentration can be determined by using standard protein content assays.
   - Dissolve the enzyme in a sufficient amount of buffer or water to obtain a ratio resin/buffer of minimum 1/4 (w/v).
   - The pH of the enzyme solution should be adjusted to the same value as the pH of the resin.

3. **Immobilization**
   - Transfer the immobilization buffer containing the enzyme into the immobilization vessel and add the Lifetech ECR base anion resin.
   - Mix the slurry for 24 h at 70 - 80 rpm.
     Note: avoid using magnetic stirring during enzyme immobilization as this can damage beads.
   - The immobilization can be performed at temperatures between 20°C - 30°C depending on enzyme stability.

4. **Filtration, washing and drying**
   - Filter the liquid phase and collect it.
   - Determine the protein content and evaluate the immobilization yield.
   - Wash the immobilized enzyme with deionized water with a suggested ratio resin/water of 1/10 (w/v) or three times with a ratio resin/water of 1/3.
   - If required, dry the immobilized enzyme.

5. **Characterization**
   - Characterize the immobilized enzyme in terms of moisture content and specific activity.

6. **Storage**
   - Transfer the immobilized enzyme into a suitable container and keep refrigerated 2°C - 8°C.
     Note: avoid freezing the immobilized enzyme since this may damage the beads.

Additional information

Particle size selection

Small particle size resins (F grade, 150 - 300 µm) are optimal for research and pharmaceutical applications if batch volumes are limited and pressure drop during filtration is not significant. F grade is also ideal for batch reactor configurations. Industrial grade (300 - 1200 µm) is optimal for large-scale application such as food enzymatic processes.

Large reaction volumes or viscous systems make large particle resins (M grade, 300 - 710 µm) optimal in fine chemical or food applications. M grade is ideal for packed bed or fluidized bed reactor configurations.

The advantage of F grade is the higher specific enzymatic activity that can be obtained, whereas M grade is easier to handle and reduces filtration time.

Drying immobilized enzymes

If reactions occur in water-free media (like organic solvents), dry immobilized enzymes may be required.

Lifetech ECR enzyme carriers can be easily dried to obtain minimized water content (< 10%). The minimum water content of an immobilized enzyme depends on the degree of minimum hydration required by the enzymes. Check if your enzyme can work in water free media. Immobilized lipases, for example, can be dried to less than 10% without any loss of activity.

Dried immobilized enzymes can be obtained using several different techniques, depending on the scale the operation is carried out and final moisture content desired. The stability of the native enzyme is another key factor that should be carefully taken in account. Stability information for the enzyme will provide an indication of the temperature that can be used to aid the drying process, or which gas can be used to remove moisture (in a fluidized bed dryer for instance).

The drying efficiency and drying time is affected by the initial moisture content of the resin after immobilization. It is important to filter the wet resin long enough to allow removal of as much liquid as possible. This can be achieved using a vacuum pump until no further liquid can be removed. The moisture content after filtration is dependent on the resin and the solvent used in the washing step. Typically, final moistures varying from 45 - 60% are obtained for different resins after filtration for water washed resins.

Table 5 summarizes some of the methods known to be used for drying of immobilised enzymes as well as their application, main advantages and/or disadvantage (Adapted from Perry's Chemical Engineering Handbook,1999).

Table 5 – Drying processes applicable to immobilised enzymes

TYPE OF DRYER	APPLICATION/COMMENT
Fluid bed dryer	Useful for small and large scale (mg scale to hundreds of kilos). Gas (typically N_2) or dry air is used to remove moisture from resin.
Vacuum dryer	Useful for pilot scale or industrial scale. Relatively fast drying time with the advantage of low temperatures. Useful for heat sensitive enzymes.
Vacuum shelf-dryer	Useful for heat sensitive product or readily oxidizable products. Potentially longer drying time. Require large space to process large amount of product.

Fluidize bed drying (FBD):

In FBD, free-flowing moist particles can be dried continuously with a residence time of a few minutes by contacting with hot or dry gases. This can be achieved at lab scale using, for instance, a syringe connected to a gas inlet (e.g. air, nitrogen or argon). This set up is frequently used to process small amounts of immobilized enzyme in 15 - 60 minutes (depending on the gas flow) achieving moisture content as low as 1%. At this scale, 1 - 10s of grams of wet material can be dried depending on the size of column (syringe) used.

The process can also be easily scaled up to bench (5 - 10 L column) or pilot/full scale (> 100 L column). Larger volumes can be processed using equipment with higher volume capacity. It is possible to fully control and monitor temperatures and moisture/humidity profile during the drying.

Vacuum drying:

The conical mixer dryer is a batch-wise operating unit commonly used in the pharmaceutical and specialty chemical industries for drying solvent or water wet, free-flowing powders. The process area is a vertically oriented conical vessel with an internally mounted screw. The dryer utilizes the heated, internal rotating screw to provide agitation of the batch improving heat and mass transfer.

The conical mixer dryer is an indirect (conduction) dryer designed to operate under full vacuum. The heating medium is hot water, steam, or thermal oil, with most applications in the temperature range of 50 -150°C and pressures in the range of 3 - 30 kPa absolute. The vapors generated during the drying process are evacuated by a vacuum pump and passed through a condenser for recovery of the solvent.

It allows time efficient drying of large quantities of product at or above pilot scale and it is ideal for heat-sensitive products, as the use of vacuum allows lower temperatures to achieve moisture removal.

Vacuum shelf dryer:

Vacuum-shelf dryers are indirect-heated batch dryers consisting of a vacuum tight chamber usually constructed of cast iron or steel plate, heated, supporting shelves within the chamber, a vacuum source, and usually a condenser.

Hollow shelves of flat steel plate are fastened permanently inside the vacuum chamber and are connected in parallel to inlet and outlet headers. The heating medium, entering through one header and passing through the hollow shelves to the exit header, is generally steam, ranging in pressure from 700 kPa gauge to sub-atmospheric pressure for low-temperature operations. Low temperatures can be provided by circulating hot water, and high temperatures can be obtained by circulating hot oil. Some small dryers employ electrically heated shelves. The material to be dried is placed in pans or trays on the heated shelves. The trays are generally of metal to ensure good heat transfer between the shelf and the tray.

Typical buffers used for enzyme immobilization

The choice of the buffer depends very much on the enzyme. As a rule of thumb, buffers in which the enzyme is stable and active are a good choice. The following buffers are typically used for enzyme immobilization:

- Phosphate
- Acetate
- Citrate
- Triethanolamine

The buffer strength is often optimized for different enzymes. High buffer concentration improves immobilization yields. A screening process is recommended in order to identify the most optimum condition.

Specific immobilization protocols and activity testing for immobilized enzymes

Procedures for immobilization of specific enzymes on specific carriers are available on request. Below and on page 15, we report on some examples for enzyme immobilization.

Lipase CALB adsorption on Lifetech ECR1030 DVB/acrylate

- Prepare a solution containing 25mg protein per gram of wet resin corresponding to 25KU TBU (tributyrin hydrolysis units) per gram of dry resin (or use ready-to-use liquid enzyme)
- Adjust pH of liquid solution of CALB Lipase to 7.5 using phosphate buffer
- Weigh 1 gram of Lifetech ECR1030M (no need to pre-treat the resin)
- Mix gently for 24h (avoid using magnetic stirring)
- Remove liquid by vacuum filtration
- Wash the resin once with sodium phosphate buffer (20 mM, pH 7.5)
- Dry the immobilized enzyme using suggested drying procedures
- Determine activity using PLU assay

Immobilized CALB activity testing (PLU activity)

Novozymes PLU method EB-SM-1069.02 is used to study the activity of CALB in the esterification reaction of 1-propanol and lauric acid. In this assay, the enzyme is incubated with neat substrates (40 mmol of each 1-propanol and lauric acid) and a small amount of water (320 µl) at 60°C. The reaction volume is 12.5 ml. The method was adapted to the equipment available in the laboratory as follows. For incubation of the reaction mixtures, an incubator shaker was used. Per reaction, 30 mg of dry immobilized CALB on ECR1030 were added, and samples were taken after 0, 10, and 20 min. 50 µl sample were diluted in 450 µl 1-heptane containing n-tridecane (111 mM) and injected (0.5 µl) into a GC. Activities were quantified with the internal standard n-tridecane. Propyl laurate units (PLU) refer to the amount (µmol) of propyl laurate formed by the enzyme in the reaction per minute.

Penicillin G acylase (PGA) covalent immobilization on Lifetech ECR8205 epoxy acrylate

- Wash 5 grams of Lifetech ECR8205 once with 0.1 M phosphate buffer pH 8.0 (ratio resin/solvent 1/1)
- Wash the resin with 1.25 M phosphate buffer pH 8.0 (ratio resin/solvent 1/1)
- Remove the liquid by vacuum filtration
- Prepare a solution containing 1500 U of PGA in 20 ml of 1.25 M phosphate buffer, pH 8.0
- Add the enzyme solution to the resin and mix for 18h at a temperature between 20°C - 25°C
- Stop mixing. Leave the mixed enzyme/resin in contact for another 20 h at room temperature. Do not stir
- Wash the biocatalyst twice with 0.02 M phosphate buffer pH 8.0 (ratio resin/solvent 1g wet/2ml) and filter
- Store the immobilized enzyme in the refrigerator

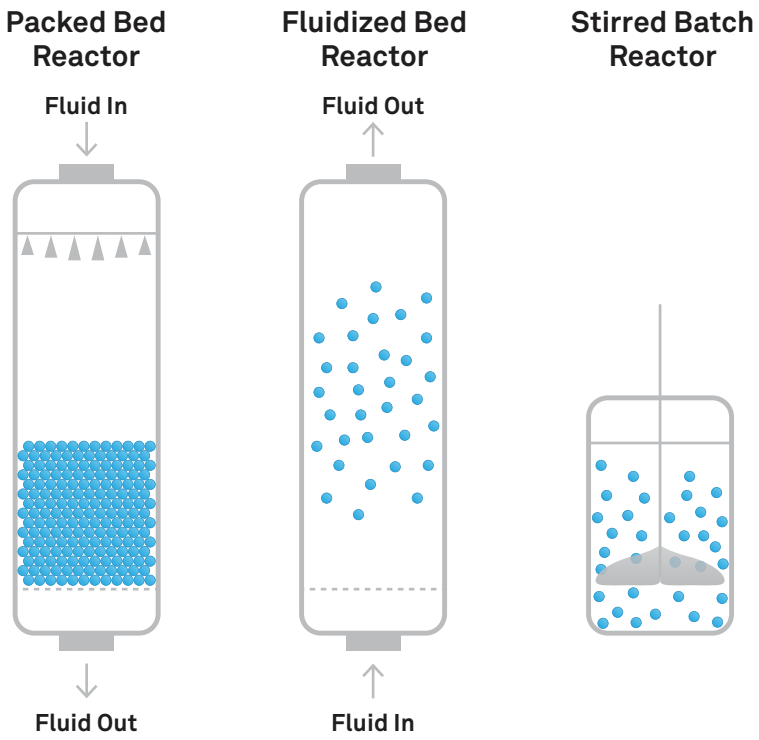
Immobilized penicillin G acylase activity test

The enzymatic activity was assayed by hydrolysis of penicillin G potassium salt. A 10% solution of benzylpenicillin in phosphate buffer (0.02M, pH 8.0) was prepared. About 30 mg (wet) of immobilized preparation were suspended in 16 ml of phosphate buffer (0.02M, pH 8.0) and 4 ml of 10% penicillin solution were added (final concentration 2%). The released phenylacetic acid was titrated, under constant stirring, with NaOH 0.05M by using an automatic titrator. One unit corresponds to the amount of preparation that hydrolyzes 1 mmol of penicillin G per min at 37°C in phosphate buffer, pH 8.0.

Bioreactors – How immobilized enzymes are used in bioprocessing

The most common enzymatic reactor for continuous operation is the packed-bed setup, a cylindrical column holding a fixed bed of catalyst particles (Figure 7). These should have a larger particle size to keep the pressure drop 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. This 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 with consequent lower pressure drop. The residence time allowed by the flow rates required for fluidization may however result in low conversion yields. Bioconversions on smaller scale are typically carried out in stirred tanks. Shear stress induced by stirring creates a hazardous environment for immobilized biocatalysts since they are prone to abrasion. Mechanical stability of enzyme carriers like Lifetech ECR is a key for optimal performance in this kind of configuration.

Figure 7 – Bioreactor configuration commonly used with immobilized enzymes



Particle size, packaging and available kits

Apart from Lifetech ECR1030 and ECR1061, which are only available in M and Full Grade, all Lifetech ECR resins are available with the following particle size range:

F Grade: 150 - 300 µm (100 - 50 mesh)

M Grade: 300 - 710 µm (50 - 25 mesh)

Full Grade: 150 - 710 µm (100 - 25 mesh)

Industrial Grade: 300 - 1200 µm (50 - 16 mesh) – for use in ionic enzyme immobilization

Lifetech ECRKIT1 enzyme carrier kit is designed for screening and evaluation purposes. This kit contains sample sizes of 50g of each resin (Table 6). Resins are supplied in their wet form.

Table 6 – Content of Lifetech ECRKIT1 enzyme carrier kit

KIT CONTENT	TYPE	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 or ionic/ (hydrophilic)
ECR8806F	Octadecyl methacrylate	Octadecyl	Adsorption
ECR1090F	macroporous styrene	None	Adsorption
ECR1030M	DVB/acrylate	None	Adsorption

References

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

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