## Lifetech









### Why Purolite®?

For over 35 years, Purolite has supplied specialty ion exchange resin technology to industries within complex regulatory environments, including biotechnology, pharmaceutical, food, fine chemical and electric power generation. Purolite is the only global company to focus 100% on resin technology.

#### **About Purolite Life Sciences**

Formed in 2012, Purolite Life Sciences provides API's, enzyme carriers and immobilized enzymes, and resins for purification and separation, to support Research and Development and production-scale applications in pharmaceuticals, protein purification, food production, bioprocessing, fine chemical and other markets.

Purolite's team of world-class researchers and scientists develop novel, high-demand and customized products to meet customer needs.

Collaborating with industry organisations and research institutes all over the world, our aim is to produce the most economical and practical resin technology products available.

Purolite has completed major refurbishments and the expansion of its Life Sciences research laboratories located in Llantrisant, South Wales UK, including the addition of a world scale agarose manufacturing plant.

Essential to life science, we have created global quality and regulatory management systems and a team of experts to ensure the highest product quality.

Purolite has a FDA inspected facility located in Romania, which has recently completed a fourth cleanroom addition. This adds to our speciality polymer capabilities with separate facilities to immobilize enzymes or ligands, backwash towers for the removal of fines, solvent or purified water washing, screening, vacuum drying and packaging. All processes are completed in a cleanroom environment with gowning and trained personnel.

In addition, Purolite is continuing its expansion in Romania with two polymer reactors located in a separate cleanroom environment for the production of various types of specialty polymers. The addition, provides Purolite with the capabilities to manufacture products through suspension polymerization, or through our patented uniform bead jetting process.

Once the products are manufactured they are functionalized, washed and packaged as required.

These expansions and additional facilities are a result of increased demand for large scale production of polymer beads ranging between 25 – 250 µm in size. This unique capability, means that commercial production can now take place on a large scale, producing beads of varying size in a short period of time

Purolite is a solutions company, taking your ideas and processes and commercialising them.



#### **Product Portfolio**





- Robust styrene/DVB resins and methacrylic resins with excellent pH and chemical stability
- Excellent pressure and flow characteristics
- Ideal for the separation of biomolecules such as proteins, amino acids, peptides & oligonucleotides



#### PuroPhase™ SPE Reverse Phase

- ▶ Wide range of Chromalite synthetic adsorbents packed in SPE columns
- Different adsorbents with a range hydrophobicity/ hydrophilicity and chemical/ physical properties
- ▶ Ideal for Solid Phase Extraction (SPE) of different analytes



#### **Praesto® Agarose Resins**

- Advanced high-flow, highly cross-linked agarose
- Excellent performance with chemical and physical stability
- ► The best Protein A and ion-exchange resins for biological protein chromatography



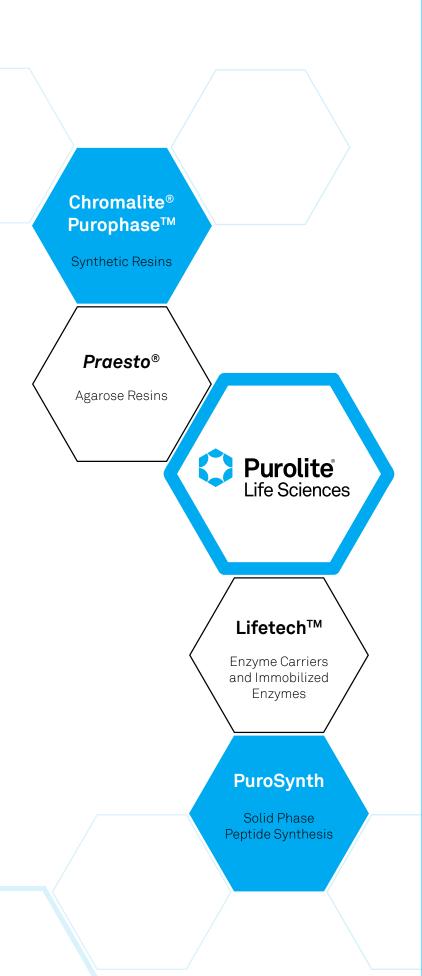
#### Lifetech™ Enzyme Carriers & Immobilized Enzymes

- Wide range of methacrylic or styrenic copolymers for immobilization of different enzymes and ready-to-use industrial immobilized lipases
- ► Superior physical, chemical and mechanical stability to allow multiple cycles
- Ideal for column or batch reactor design



#### Pharmaceutical APJs & Excipients - Purolite

- Wide range of functionalised synthetic acrylic or styrene resins
- Manufactured to meet the requirements of both the US Food and Drug Administration (FDA) and the European Union
- Used for a range of pharmaceutical applications including reducing the blood cholesterol levels, hyperkalemia and taste/odour control



Leading innovation. Exceeding expectations.

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### Purolite and SpinChem® Collaboration

#### **Background**

Thanks to a collaboration between SpinChem® and Purolite, we can offer an innovative packed solution that will allow for easier and faster screening and optimization of biocatalysis production processes.

SpinChem® magnetic rotating bed reactor (MagRBR) is designed for parallel laboratory development and reaction screening in 5 - 100 mL volumes.

SpinChem® MagRBR is delivered pre-packed with a variety of Purolite® Lifetech™ ECR enzyme carrier resins and immobilized enzymes.

#### SpinChem® MagRBR

The SpinChem® MagRBR is a pre-packed rotating bed reactor for milliliter scale reactions, but can be scaled up to 100 liter scale if required.

By including a magnet centrally in a 1 mL polyamide cylinder, the rotational forces can be exploited on a magnetic stirrer plate. The resin contained within the filter experiences excellent contact with the solution as it is pushed through the packed resin bed by the centrifugal forces created as the MagRBR rotates.

Due to inertial forces, spinning the SpinChem® MagRBR in substrate solution will allow the substrate to have multiple passages through the MagRBR to interact with the enzyme immobilized on the resin within.

The great advantage of SpinChem® MagRBR is the simplicity of handling and the easy process setup. The reaction progress can be monitored easily for kinetics determination, and at the end of the process the MagRBR can be removed from reaction with minimal effort. After completing the screening process, the MagRBR is easily washed and removed (see recycling experiments) to be reused at a later time, or in another application.



## **SpinChem® MagRBR Features**

Size	H 7.3 mm x D 18mm		
Enzyme Content	0.5 mL corresponding to 150 - 200 mg *		
Container Material	Polyamide (PA)		
Filter Porosity	44 μm		
Operating Temperature Limits (°C)	- 10°C - + 50°C		
Supplied as	Dry **		
Total Moisture	0 - 5 % **		
Optimal Storage Conditions	2 - 20°C		
Expiry Date (from date of manufacture)	1 year		
Chemical Resistance	Strong Acids		
	Weak Acids	<b>✓</b>	
	Alcohols	<b>✓</b>	
	Alkalis	<b>✓</b>	
	Aromatic Hydrocarbons	<b>✓</b>	
	Halogens		
	Halogenated Solvents		
	Hydrocarbons and Oils	~	
	Organic Solvents	<b>✓</b>	
	Oxidizing Agents		

<sup>\*</sup> Exact quantity stated on packaging

<sup>\*\*</sup> MagRBR containing Epoxy300 are supplied in IPA

### Recommended Equipment for use of SpinChem® MagRBR

SpinChem® MagRBR is designed with an internal magnet and can be used with standard magnetic plate stirrers and flat-bottomed tubes or vials of diameter >2 cm.

To streamline the workflow and assure optimal results, everything needed to get your screenings up and running is included in the SpinChem® MagRBR starter kit. The SpinChem® MagRBR starter kit includes a MagRBR screening kit of your choice, reaction tubes, magnetic stirrer, stirring control unit and tube rack.

#### 1. SpinChem® reaction tube

Transparent, flat base tube in polypropylene for liquid volumes up to 23 mL. Included with MagRBR.

#### 2. SpinChem® tube rack

Tube rack in stainless steel with sockets for docking the six position magnetic stirrer, and handles for lifting.

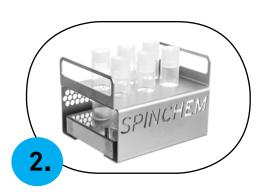
#### 3. SpinChem® magnetic stirrer

Waterproof, six position magnetic stirrer, suitable for water baths, oil baths, incubators and ovens up to +50 °C.

#### 4. SpinChem® stirring drive control unit

Control unit for magnetic stirrer, that allows stirring rates up to 2000rpm, and automatic memory function for last used settings.









## Suggested Immobilization Protocol using SpinChem® MagRBR containing Lifetech™ ECR Resins

#### Overview

This section contains general information and guidelines regarding the handling of SpinChem® MagRBR containing Lifetech™ ECR resins. Lifetech™ ECR resins have been specifically designed for enzyme immobilization and contain different functional groups to support specific types of enzyme immobilization. Depending on the enzyme to be immobilized, and the type of immobilization desired, different enzyme immobilization protocols can be applied.

#### **Example Immobilization Procedure**

#### 1. Resin equilibration

SpinChem® MagRBR filled with ECR resins are supplied in dry form except for Epoxy300, which is supplied in IPA. No wetting is necessary for Epoxy300 MagRBR, however the IPA shipping solvent should be removed before use. For the rest of the Lifetech ECR resions, wetting may be necessary depending on the immobilization approach. Wet MagRBR by spinning in minimum 5 mL 20 % ethanol (500 rpm, ambient temperature) for 2 - 24 hours.

#### 2. Enzyme solution

Consider a protein loading of 50 - 100 mg protein per gram of wet resin. Use a minimum of 5 mL of liquid enzyme solution prepared in water or suitable buffer. Therefore, ensuring the submersion of the whole MagRBR in liquid.

#### 3. Immobilization on hydrophobic and epoxy ECR resins

Following the equilibration procedure, wash MagRBR with 2 x 5 mL of dH2O or suitable buffer solution (500 rpm stirring, minimum 5 min), remove washing liquid and add the enzyme solution. Stir the MagRBR at 500 rpm at ambient temperature for 24 hours, and withdraw liquid samples when required for analysis of protein binding. When immobilization is completed, remove MagRBR from enzyme solution and rinse in dH2O or suitable buffer.

#### 4. Immobilization on amino-functionalised ECR resin

MagRBR filled with amino-functionalised ECR resin require pre-activation with glutaraldehyde. Wash MagRBR with 2 x 5 mL of potassium phosphate buffer (0.02 M, pH 8, 500 rpm stirring, minimum 5 min), remove washing solution, then incubate in 5 mL of 2 % glutaraldehyde solution in potassium phosphate buffer (0.02 M, pH 8, 500 rpm stirring) for 1 hour. Wash the MagRBR with 4 x 5 mL of dH2O (500 rpm stirring, minimum 5 min), followed by 4 x 5 mL of suitable immobilization buffer. Remove washing solution, then add the enzyme solution prepared as per point 2. Stir the MagRBR at 500 rpm at ambient temperature for 24 hours, and withdraw liquid samples when required for analysis of protein binding. When immobilization is completed, remove MagRBR from enzyme solution and rinse in dH2O or suitable buffer.

#### **Storage**

Transfer the MagRBR with immobilized enzyme into a suitable container and keep refrigerated at 2 - 8°C.

Note: Avoid freezing the immobilized enzyme since this may damage the beads, and subsequently compromise the integrity of the MagRBR filter.

# Effect of Wetting SpinChem® MagRBR Filled with Lifetech™ ECR before Enzyme Immobilization

One of the key benefits of MagRBR is the simplicity of recycling once the enzyme has been immobilized onto the contained resin. Depending on the nature of the solution used for the biocatalytic reaction, different washing solutions can be used for the MagRBR.

#### **Biocatalysis in Organic Media:**

Wash MagRBR with  $3 \times 5$  mL suitable organic solvent at 500 rpm for 5 minutes per wash. When possible, the solvent participating in the reaction can be used. When using immobilized lipases, heptane can be used as a washing solvent. Alternatively, ethanol or IPA could be considered. After washing, allow to dry before returning to cold storage.

#### **Biocatalysis Aqueous Media:**

Wash MagRBR with 3  $\times$  5 mL deionised water at 500 rpm for 5 minutes per wash and return directly to cold storage.

## Effect of wetting SpinChem® MagRBR filled with Lifetech™ ECR before enzyme immobilization

SpinChem® MagRBR are supplied filled with resin in dry form. While MagRBR containing immobilized enzyme (CalB immo kit and Lipase immo kit) can be used as supplied, MagRBR containing ECR resins for immobilization may require wetting for better mass transfer. The results described here show the effect of wetting SpinChem® MagRBR filled with ECR1090M, ECR1030M and ECR8806F resins before lipase CalB immobilization by hydrophobic interaction. On all three hydrophobic ECR resins tested, immobilization was more efficient when resins were preliminary wetted (see procedure described in immobilization protocol) with all enzyme adsorbed in 24 hours. On the contrary when SpinChem® MagRBR were used without wetting, the immobilization was not efficient even after 24 hours spinning.

Immobilization on wetted ECR8806 was exceptionally fast with all the enzyme adsorbed after 1 hour.

Figure 1. Lipase CalB Activity

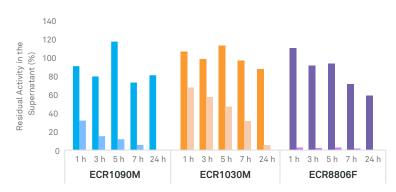


Figure 1. Lipase CalB activity of immobilization supernatant over a 24-hour immobilization process, as a percentage of the initial enzyme solution activity. Dark columns represent data for the dry MagRBR as supplied, light columns represent data for wetted MagRBR following the wetting protocol. Immobilization process followed: hydrophobic interaction as detailed in Purolite Enzyme Immobilization Guide.

In this experiment, the effect of wetting is very different between hydrophobic and hydrophilic ECR resins. ECR8204F is a hydrophilic epoxy methacrylate, and ECR8309F is a highly hydrophilic amino methacrylate and there is no significant difference in performance between the dry and wet resin. By comparison, ECR8285 is a hydrophobic epoxy/butyl methacrylate that requires a long time to wet. This explains the difference in performances between the dry and wetted ECR8285, which performs very similarly to ECR8806F.

Figure 2. Lipase Activity - Epoxy and Amino Resins

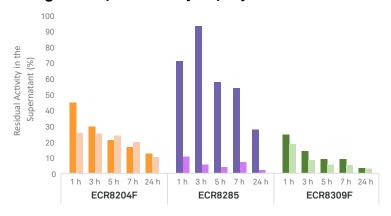


Figure 2. Lipase activity of immobilization supernatant over a 24 hour immobilization process on epoxy and amino resins, as a percentage of the initial enzyme solution activity. Dark columns represent data for the dry MagRBR as supplied, light columns represent data for wetted MagRBR following the wetting protocol.

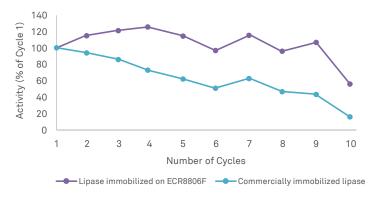
#### Recycling studies on SpinChem® MagRBR

Successful recycling experiments have been conducted using immobilized lipase transesterification of methyl stearate into methyl caprylate. Washing of the MagRBR was conducted by submersion in 3 x 5 mL heptane with magnetic stirring at 500 rpm for 5 minutes each to remove residual substrate and product.

To verify the stability of SpinChem® MagRBR upon multiple cycles, a comparative test was done using SpinChem® MagRBR filled with a commercial immobilized lipase, and SpinChem® MagRBR filled with ECR8806F and subjected to enzyme immobilization.

Results show that enzyme immobilization on ECR8806F gives a very stable preparation that can be re-used for up to 10 cycles without loss of activity. The retention of activity is markedly higher than that observed for the commercial preparation and indicates the efficiency of the immobilization using MagRBR.

#### Figure 3. Stability of Biocatalyst Activity

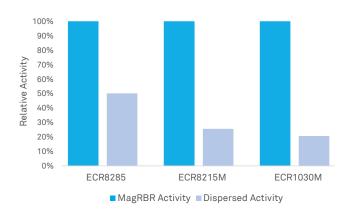


**Figure 3.** Stability of biocatalyst activity over repeat cycles of transesterification using immobilized lipase in SpinChem® MagRBR.

## Effect of mass transfer on SpinChem® MagRBR compared with dispersed resin

The effect of spinning and mass transfer in MagRBR is evident when comparing the retained activity of lipase immobilized on different carriers as ECR1030M, ECR8285 and ECR8215M. When a lipase is immobilized on ECR contained in a SpinChem® MagRBR, the activity obtained is much higher than when using standard immobilization procedures (Figure 4). This benefit is due to improved mass transfer in the MagRBR, owing to the rotational forces being exploited.

Figure 4. Comparison of Enzyme Activities



**Figure 4.** Comparison of enzyme activity obtained after immobilization of a lipase on ECR8285 (covalent immobilization, hydrophobic), ECR8215M (covalent immobilization, hydrophobic) and ECR1030M (adsorption, hydrophobic).

## **Ordering Information**

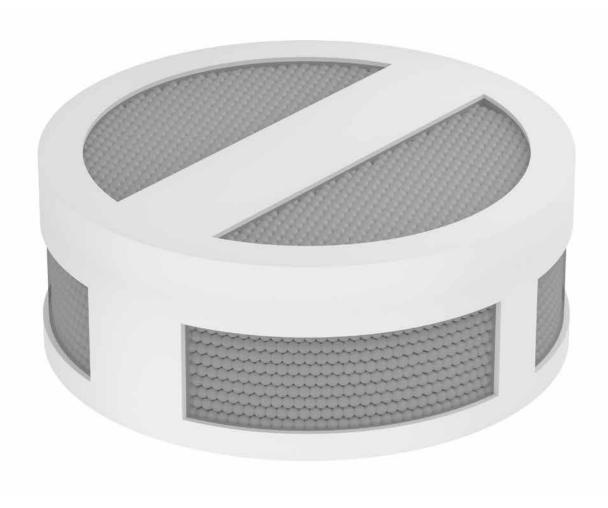
## **Ordering Information**

To place your order, simply contact the relevant regional office via email or telephone using the information on the back page of this brochure, and quote your order number from the tables on the following pages.

If you wish to discuss your purification challenges with a specialist, we have dedicated experts on-hand across the globe to provide knowledgeable, same-day technical assistance.

SpinChem® MagRBR				
PRODUCT	PACK SIZE	ORDER NUMBER		
SpinChem® MagRBR M10 CalB immo KIT	6 MagRBR	LS02000-MKIT		
SpinChem® MagRBR M10 Lipase immo KIT	6 MagRBR	LS02001-MKIT		
SpinChem® MagRBR M10 ECRKIT1 KIT	6 MagRBR	LS01001-MKIT		
SpinChem® MagRBR M10 ECR1090F	6 MagRBR	LS01100-MKIT		
SpinChem® MagRBR M10 ECR1090M	6 MagRBR	LS01105-MKIT		
SpinChem® MagRBR M10 ECR1091M	6 MagRBR	LS01115-MKIT		
SpinChem® MagRBR M10 ECR8204F	6 MagRBR	LS01211-MKIT		
SpinChem® MagRBR M10 ECR8204M	6 MagRBR	LS01215-MKIT		
SpinChem® MagRBR M10 ECR8209F	6 MagRBR	LS01231-MKIT		
SpinChem® MagRBR M10 ECR8209M	6 MagRBR	LS01235-MKIT		
SpinChem® MagRBR M10 ECR8215F	6 MagRBR	LS01241-MKIT		
SpinChem® MagRBR M10 ECR8215M	6 MagRBR	LS01245-MKIT		
SpinChem® MagRBR M10 ECR8285	6 MagRBR	LS01269-MKIT		
SpinChem® MagRBR M10 ECR8309F	6 MagRBR	LS01351-MKIT		
SpinChem® MagRBR M10 ECR8309M	6 MagRBR	LS01355-MKIT		
SpinChem® MagRBR M10 ECR8315F	6 MagRBR	LS01361-MKIT		
SpinChem® MagRBR M10 ECR8315M	6 MagRBR	LS01365-MKIT		
SpinChem® MagRBR M10 ECR8409F	6 MagRBR	LS01381-MKIT		
SpinChem® MagRBR M10 ECR8409M	6 MagRBR	LS01385-MKIT		
SpinChem® MagRBR M10 ECR8415F	6 MagRBR	LS01391-MKIT		
SpinChem® MagRBR M10 ECR8415M	6 MagRBR	LS01395-MKIT		
SpinChem® MagRBR M10 ECR8806F	6 MagRBR	LS01411-MKIT		

SpinChem® MagRBR		
PRODUCT	PACK SIZE	ORDER NUMBER
SpinChem® MagRBR M10 ECR8806M	6 MagRBR	LS01415-MKIT
SpinChem® MagRBR M10 ECR1030M	6 MagRBR	LS01125-MKIT
SpinChem® MagRBR M10 ECR1061M	6 MagRBR	LS01135-MKIT
SpinChem® MagRBR M10 ECR1508	6 MagRBR	LS01502-MKIT
SpinChem® MagRBR M10 ECR1504	6 MagRBR	LS01512-MKIT
SpinChem® MagRBR M10 ECR1604	6 MagRBR	LS01552-MKIT
SpinChem® MagRBR M10 ECR1640	6 MagRBR	LS01562-MKIT
SpinChem® MagRBR M10 Epoxy300	6 MagRBR	PR01270-MKIT



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