

Enzyme immobilization screening using magnetic rotating bed reactors

The SpinChem® magnetic rotating bed reactor (MagRBR) has been proven to be a useful tool in screenings and evaluations of materials used in enzyme immobilizations. In this application note, the SpinChem® MagRBR ECR screening kit, pre-packed with Purolite® Lifetech™ resins, was used to screen six different enzyme carrier resins in parallel for the immobilization of lipase CalB. Easy sampling and monitoring of the process, together with effortless handling, established the MagRBR as a time and labour efficient screening device.

Keywords: Immobilized enzymes, Rapid Screening, Biotransformation, Easy handling

Immobilization of enzymes offers straightforward recyclability and removal of enzyme from processed solution. This procedure may also confer increased thermal and operational stability to the enzyme. For immobilization of enzymes on solid-phase beads, the immobilization principles can broadly be divided into covalent and non-covalent interactions. Many immobilization chemistries are commercially available, such as epoxy, iodoacetyl, amino, azlactone and hydrophobic, and the method chosen depends largely on the properties of the enzyme in question.

Finding the optimal chemistry and solid-phase material for immobilization of enzymes relies heavily on trial-and-error. The right resin will ensure satisfactory immobilization yield, as well as high activity and stability of the enzyme. SpinChem's magnetic rotating bed reactor (MagRBR) is designed for screening of immobilized biocatalysts and enzyme immobilization resins in liquid volumes of 5-100 mL. Through a collaboration with Purolite® Life Sciences, the MagRBR is delivered pre-packed with a wide range of resins. As the MagRBR is spun in solution, the centrifugal forces created by the rotary movement will force the solution through the packed bed of resin repeatedly. The SpinChem® MagRBR enzyme carrier resin (ECR) screening kit consists of six MagRBRs filled with different ECR resin for fast and convenient parallel testing of immobilization materials.



Fig 1. SpinChem® MagRBR starter kit, including six MagRBRs pre-packed with resin, reaction tubes, six-position magnetic stirrer, stirrer control unit and tube rack.

In this application note, the SpinChem® MagRBR enzyme ECR screening kit was used to identify the resin best suited for immobilization of the proof-of-concept enzyme *Candida antarctica* lipase B (CalB). The six MagRBRs included in the ECR screening kit were run in parallel on a six-position magnetic stirrer plate. Each MagRBR contained 0.5 mL of ECR carrier resin, and was spun in enzyme supernatant. To monitor the immobilization process, enzymatic activity in the supernatant was measured at five points during a 24 h timespan to determine the amount of residual enzyme in the solution.

All six resins evaluated in this study succeeded in immobilizing the enzyme CalB to some extent, ranging from just over 30% to nearly complete immobilization. The resin best suited was ECR8806F (octadecyl methacrylate macroporous) having adsorbed 97% of the total amount of enzyme after 1 h.

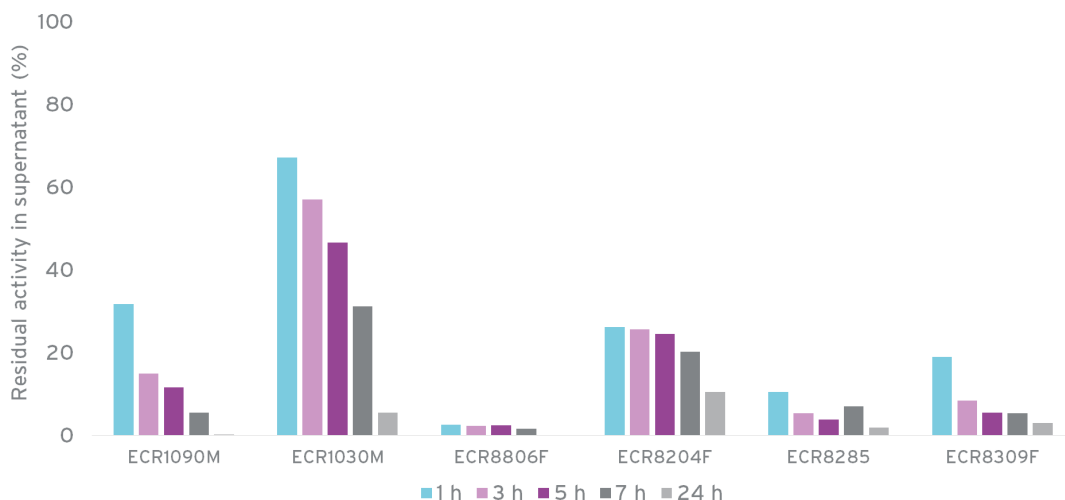


Fig 2. Graphs showing CalB residual enzyme activity in the supernatant over 24 h, as percentages of initial enzyme activity. The enzyme solution was prepared for each type of resin according to the protocols outlined in the SpinChem guidelines for enzyme immobilization (available at www.spinchem.com/support). The resins screened were ECR1090M, ECR1030M, ECR8806F, ECR8204F, ECR8285 and ECR8309F (all Purolite® Lifetech™). The enzymatic activity of samples taken from the supernatant was determined using a lipase activity test based on the transformation of *p*-nitrophenylbutyrate to *p*-nitrophenol and butyric acid. The reaction was quantified by the use of a spectrometer through an increase in absorbance at 450 nm due to the conversion into the products. ECR8806F was determined to be the best candidate, immobilizing 97% of the total amount of enzyme within 1 h.

Utilizing the efficient mass transfer and easy handling of the SpinChem® RBR technology, proved to be a simple and efficient way of screening for suitable enzyme immobilization resins.

As the resin is contained within the MagRBR throughout the screening, sampling and monitoring of the process

was fast and convenient as no filtration steps were required prior to analysis. The convenient format enables further screening efficiency, by simply increasing the number of MagRBRs run in parallel. After successful resin screening, the MagRBR format is also offered containing a single resin of interest, for further evaluations of parameters such as pH, temperature, buffer type, substrate-to-enzyme ratio and substrate scope.

Conclusions:

- The SpinChem® MagRBR ECR screening kit is a simple and convenient tool for enzyme immobilization screenings.
- As no filtration steps are required, resin screening is easily sampled and monitored.
- Once immobilized, the MagRBR allows for easy washing and recycling of enzymes.

Resin	Material	Particle size (µm)	Pore size (Å)	Stable pH range
ECR8204F	Epoxy methacrylate	150-300	300-600	5-9
ECR8309F	Amino methacrylate	150-300	600-1200	3-10
ECR8285	Epoxy/butyl methacrylate	100-710	400-600	5-9
ECR8806F	Octadecyl methacrylate	150-300	500-700	2-10
ECR1090M	Polystyrenic	300-710	900-1100	1-14
ECR1030M	DVB/methacrylate	300-710	220-340	1-14

Fig 3. Table of properties for ECR resins included in the SpinChem® MagRBR ECR screening kit.



The SpinChem® rotating bed reactor (RBR) technology is revolutionizing mass transfer in heterogeneous reactions where solid phases are used for catalysis, enzymatic reactions, adsorption, scavenging and other processes. The convenience of a protected bed within an RBR significantly reduces the need for post-reaction work-up. The SpinChem® RBR concept is fully scalable from laboratory to production, thus providing both more efficient reaction development and improved production economy.

Products: SpinChem® MagRBR ECR screening kit (5101-230), SpinChem® MagRBR starter kit (9100-001)

