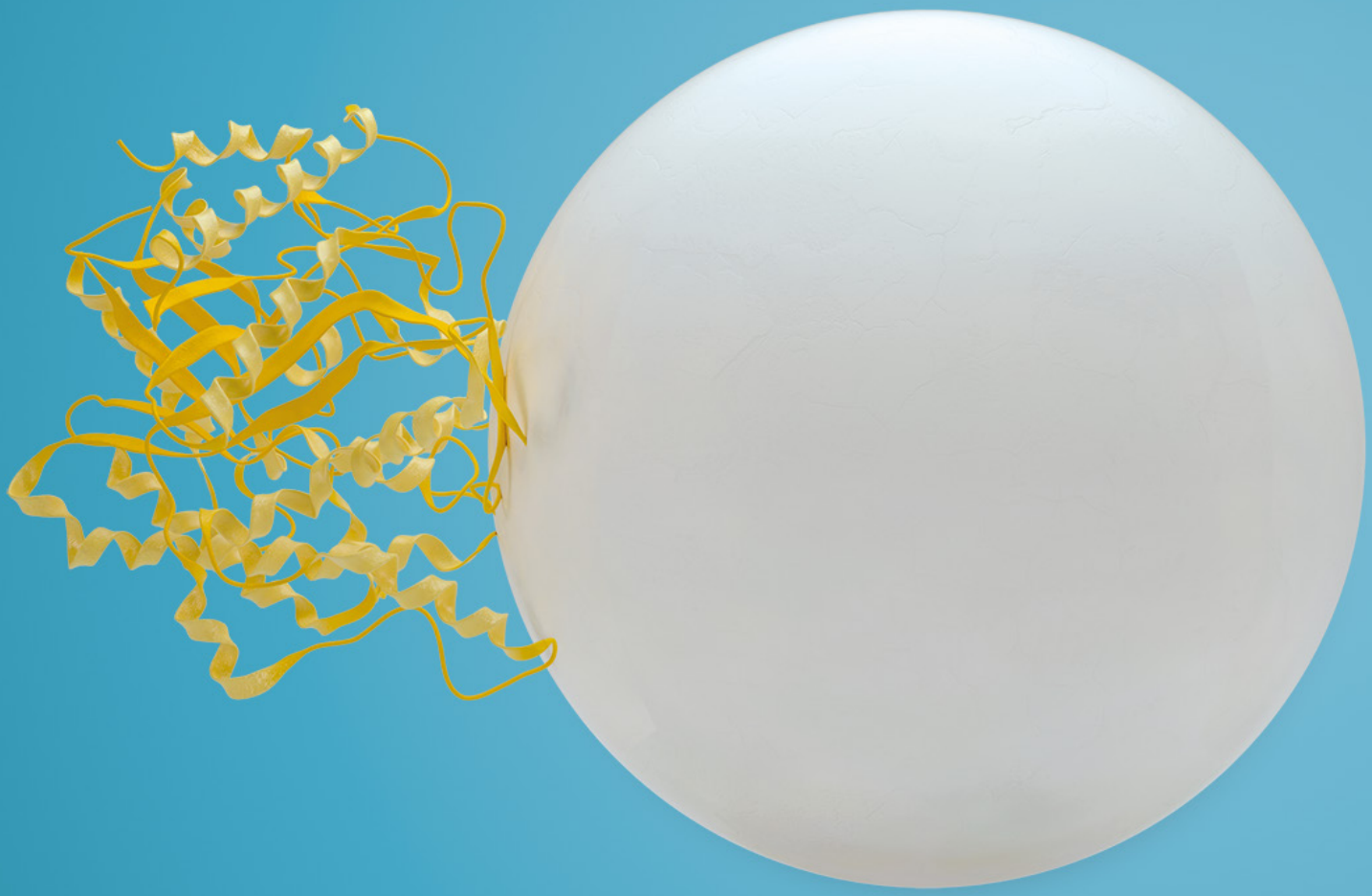
Enzyme Immobilization Procedures



Lifetech™ ECR

for ionic, covalent or adsorption-based enzyme immobilization

Chromalite® MIDA

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

Purolite® Life Sciences

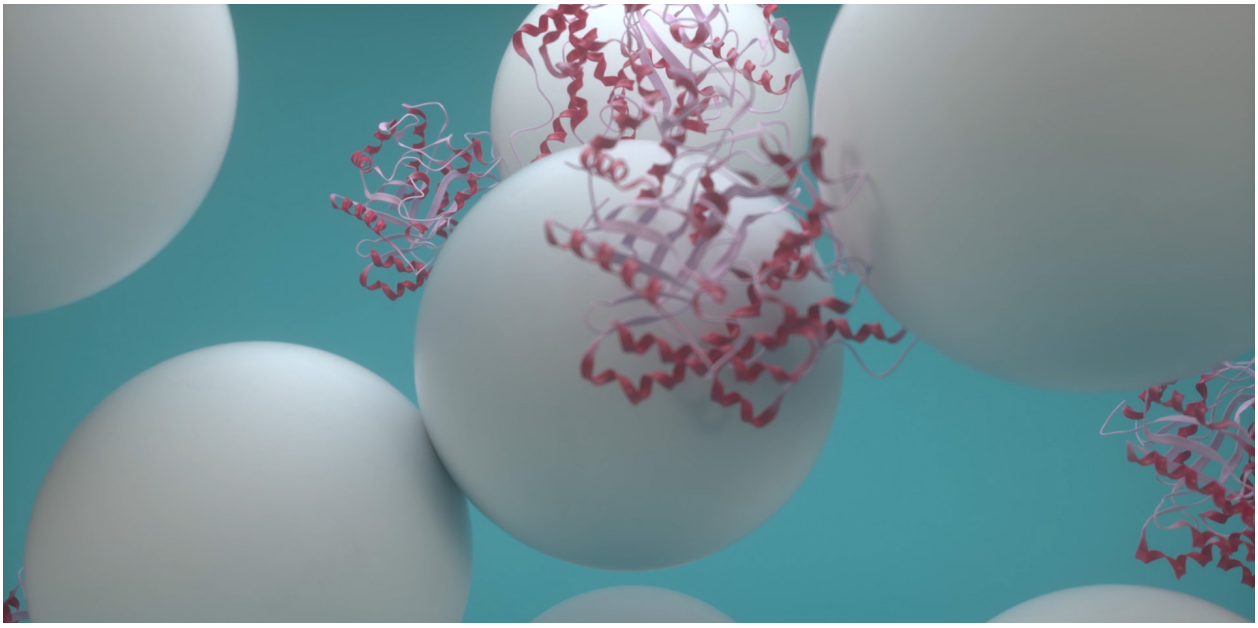
Enzyme immobilization resins for applied and industrial biocatalysis

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 intense multipoint covalent binding for minimal enzyme leakage. Immobilization via ionic interaction/adsorption 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 guidance on protocols for enzyme immobilization, and is designed to aid you in achieving optimal results from your Purolite Life Sciences resin. If you have any further queries, please contact lifesciences@purolite.com for expert technical support.



Contents

General guidelines for all immobilization procedures

- The enzyme for immobilization can be a native enzyme in liquid or solid form (i.e. lyophilized).
- Buffer solutions should be compatible with enzyme activity and stability.
- Avoid using buffers with amino-containing reagents such as tris or ethanolamine.
- When incubating the resin with solutions for activation, washes and immobilization, a resin:buffer ratio of 1:4 (w/v) is recommended. Mix the slurry gently, avoiding foam formation.
  Note: *Avoid using magnetic stirring as this can damage the beads.*
- Remove liquids and/or solutions by filtration.
- All steps can be performed at temperatures of 20 - 25°C, depending on enzyme stability.
- Consider a protein loading of approx. 50 mg protein per gram of wet resin. Protein concentration can be quantified by using standard protein determination assays. Dissolve the enzyme in buffer to obtain a ratio resin:buffer of 1:4 (w/v).
- General enzyme properties, (e.g. isoelectric point value) can be obtained in databases such as Brenda (www.brenda-enzymes.org)

After **every** immobilization process:

- Filter the liquid phase, collect it and determine the protein content in the liquid for immobilization yield.
- Wash resin with immobilization or washing buffer and remove excess of liquid by filtration.
- Characterize the immobilized enzyme in terms of moisture content and specific activity.
- Remove excess liquid and transfer the immobilized enzyme into a suitable container and keep refrigerated between 2 - 8°C.
- Avoid freezing the immobilized enzyme as this may damage the beads.
- If required, dry the immobilized enzyme by vacuum or in fluidized bed ensuring the temperature is suitable for enzyme stability.

Suggested equipment & consumables for small-scale enzyme immobilization

Example	Volume / specifications	Component	Product code	Recommended resin quantity (g)
Supelco® / Merck immobilization cartridges*	6 mL	Tube	57242	0.5 - 1.5
		Frit	57181	
		Cap	52173-U	
	12 mL	Tube	57179	1.5 - 3
		Frit	57183	
		Cap	52174-U	
	60 mL	Tube	57022	3 - 10
		Frit	57184	
		Cap	52176-U	
	6-60 mL	Female luer	57098	
Supelco® / Merck vacuum manifold*			57250-U	
Cole-Parmer® rotary mixer	Min. 20 rpm	Stuart tube rotator (SB2/SB3)	UY-07650-11	

* available from https://www.sigmaaldrich.com/united-kingdom.html

Immobilization procedure | Lifetech™ Epoxy-functionalized resins

General guidance

- Immobilization on epoxy resins is more efficient when using highly concentrated buffers (approx. 1M or higher). However, buffers at concentrations >1M are difficult to apply in industrial conditions due to limited solubility of salts.
- For desorption of non-covalently bound enzymes from the support, either use washing buffers with a strength of 0.01 - 0.05 M or deionized water.

Procedure

1. Resin equilibration

- Wash the resin with immobilization buffer and remove excess liquid (filter). A resin : buffer ratio of 1:2 (w/v) is preferable. Repeat the process 2 - 4 times.

2. Preparation of the enzyme solution

- Dissolve the native enzyme (liquid or solid) immobilization buffer.
- 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 1:2 - 1:4).

3. Immobilization

- Transfer the Lifetech™ ECR Epoxy resin to the immobilization vessel and add the immobilization buffer containing the enzyme.
- Mix the slurry gently for 18 hours. Stop after 18 hours and leave without mixing for another 20 hours.
- Do not perform immobilizations at high temperatures (> 30°C as this can cause degradation of the epoxy rings (hydrolysis) and facilitate microbial growth.

4. Filtration, washing and drying

- Wash the resin with washing buffer. Repeat process 2 - 4 times, under gentle mixing or in column wash.
- Remove the excess of liquid by filtration.
- If required, dry the immobilized enzyme.

Immobilization procedure | Lifetech™ Amino-functionalized resins

General guidance

- Immobilization on amino resins is most efficient when using buffers of low concentration (0.01 - 0.05 M)
- Use washing buffer for desorption of non-covalently bound enzymes from the support (0.01 - 0.05 M containing up to 0.5 M NaCl)

Procedure

1. Resin equilibration

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

2. Preparation of 1% glutaraldehyde buffer

- Starting from a concentrated solution of 25% or 50% glutaraldehyde, prepare a 1% glutaraldehyde (v/v) solution using the immobilization buffer. Use the 1% glutaraldehyde solution immediately.

3. Pre-activation of the amino resin

- Add the 1% glutaraldehyde buffer to the resin. The optimal volume of 1% 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 - 25°C.
- Filter and wash the beads with immobilization buffer using a resin: buffer ratio of 1:4 (w/v). Repeat 2 - 4 times. Beads are then ready for the enzyme immobilization step.
  Note: *Avoid storing pre-activated resin for a period longer than 48 hours.*
  Note: *A change in color of the beads (orange-brown) may occur and is normal.*

4. Prepare enzyme solution

- Dissolve the native enzyme (liquid or solid) in immobilization buffer.
- 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 1:2 - 1:4).

5. Immobilization

- Transfer the Lifetech™ ECR pre-activated amino resin (from step 3) to the immobilization vessel and add the enzyme solution (from step 4).
- Mix the slurry gently for 18 hours maintaining the beads in suspension.
- The immobilization can be performed at 20 - 25°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.

6. Filtration, washing and drying

- Filter the liquid phase.
- Wash the resin with immobilization buffer.
- An additional washing step using the buffer containing 0.5 M NaCl for complete desorption of non-covalently bound proteins is recommended, followed by a wash with the immobilization buffer

Immobilization procedure | Lifetech™ Adsorbent resins

General guidance

- For Lifetech™ ECR Macroporous or Octadecyl resins
- Use a low molarity immobilization buffer (0.01 - 0.05 M or deionized water)

Procedure

1. Resin equilibration

- Wash the resin with immobilization buffer and remove liquid excess. A resin:buffer ratio of 1:2 (w/v) is preferable; repeat 2 - 4 times.

2. Preparation of the enzyme solution

- Dissolve the native enzyme (liquid or solid) in immobilization buffer.
- Dissolve the enzyme in buffer solution to obtain a 1:4 (w/v) resin:buffer ratio. Low molarity buffers or water can be used for this type of immobilization.

3. Immobilization

- Transfer the Lifetech™ ECR resin to the immobilization vessel and add the enzyme solution.
- Mix the slurry for 24 hours ensuring the beads are in suspension.
- The immobilization can be performed at temperatures of 20 - 25°C depending on enzyme stability.

4. Filtration, washing and drying

- Filter the liquid phase and collect it.
- Wash the resin with washing buffer (ratio resin:buffer of 1:4 (w/v), repeat 2 - 4 times.
- Depending on the final application, adsorbed immobilized enzymes may need to be dried before use.

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*

Immobilization procedure | Lifetech™ Ionic immobilization resins

General guidance

- Use a low molarity immobilization buffer (0.01 - 0.05 M or deionized water)
- Consider the isoelectric point (pI) of the enzyme and operative pH for buffer selection
- The enzyme should be negatively charged and the immobilization should be carried out at a pH of 1 - 2 units higher than the isoelectric point of the enzyme

Procedure

1. Resin equilibration

- Equilibrate the resin in deionized water and adjust the pH using 1M HCl or 1M NaOH to a value 1 - 2 units above the pI of the enzyme; the use of a pH titrator might be beneficial. Ensure the pH remains stable for over 30 minutes.
- Filter the resin after pH adjustment to remove liquid excess.

2. Preparation of the enzyme solution

- Dissolve the native enzyme (liquid or solid) in immobilization buffer.
- Dissolve the enzyme in a sufficient amount of buffer 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 Lifetech™ ECR amino resin to the immobilization vessel and add the immobilization buffer containing the enzyme.
- Mix the slurry for up to 24 hours ensuring the beads are in suspension.

4. Filtration, washing and drying

- Wash the immobilized enzyme with deionized water with a suggested ratio resin:water of 1:4 (w/v) and repeat three times.

Immobilization procedure | Chromalite® MIDA for affinity immobilization

General guidance

- Metal loading solution: use divalent metals such as Ni^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+} chloride or sulfate salts. The SpectraChrom KIT provides resins pre-loaded with metal.
- Immobilization buffer: use a buffer that is compatible with enzyme activity and stability with a recommended concentration of up to 0.1M NaCl. Avoid buffers containing more than 50mM imidazole or chelating chemicals such as EDTA. If the selectivity towards the tagged protein is low, add up to 50 mM imidazole to the immobilization buffer. Ensure pH remains between 4 and 9.
- Washing buffer for desorption of non-affinity bound enzyme from the support is the same as your immobilization buffer, but if the selectivity towards the tagged protein is low, add up to 50 mM imidazole.

Immobilization procedure

1. Resin equilibration

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

2. Metal loading

- Use a ratio of 0.3 mmol metal per gram of wet resin.
- Prepare a 0.1 M solution of metal salt in water. Sulfate or chloride salts will work equally.
- Add the metal solution to wet resin using a resin:solution ration of 1:3 (w/v).
- Incubate under mixing for 3 hours at room temperature (avoid magnetic stirring).
- Remove liquid by filtration and wash the resin 4 times with water or immobilization buffer. Use a ratio 10ml washing solution per 1g wet resin.
- For metal loading in column, the same loading conditions could be applied ensuring good metal solution recirculation. It is also possible to wash the unbound metal in column using water or immobilization buffer.

3. Prepare enzyme solution

- Dissolve the native enzyme (liquid or solid) in immobilization buffer.
- Dissolve the enzyme in buffer to obtain a ratio resin:buffer of 1:4 (w/v).

4. Immobilization

- Transfer the enzyme solution into the immobilization vessel containing the metal loaded resin.
- Mix the slurry for 3 - 5 hours ensuring the beads are in suspension. The immobilization can be performed at temperature 20 - 25°C or accordingly to enzyme stability.

5. Filtration, washing and drying

- Filter the liquid phase.
- Wash the resin with immobilization buffer 3 - 4 times. An additional washing step using a 0.5 M NaCl containing buffer for complete desorption of non-affinity bound proteins is recommended.
- To improve the washing of non-tagged proteins, up to 50 mM imidazole can be added to the washing buffer – the imidazole concentration should be determined experimentally, so the enzyme of interested is not removed.
- If required, dry the immobilized enzyme by vacuum or in fluidized bed; ensure the drying temperature is suitable for enzyme stability.

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Americas
2201 Renaissance Boulevard
King of Prussia, PA
19406

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

www.purolitelifesciences.com

P-000026-112020-R1-PLS