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# Sunresin Seplife® EMC resins

## Enzyme Immobilization Procedures

BIOCATALYSIS

**August 2024**

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## Sunresin Seplife® EMC enzyme carriers

Seplife® EMC resins are robust enzyme carriers designed for research and industrial applications. The variability in functional groups, porosities, matrices, and particle sizes offers the widest toolbox for enzyme immobilization.

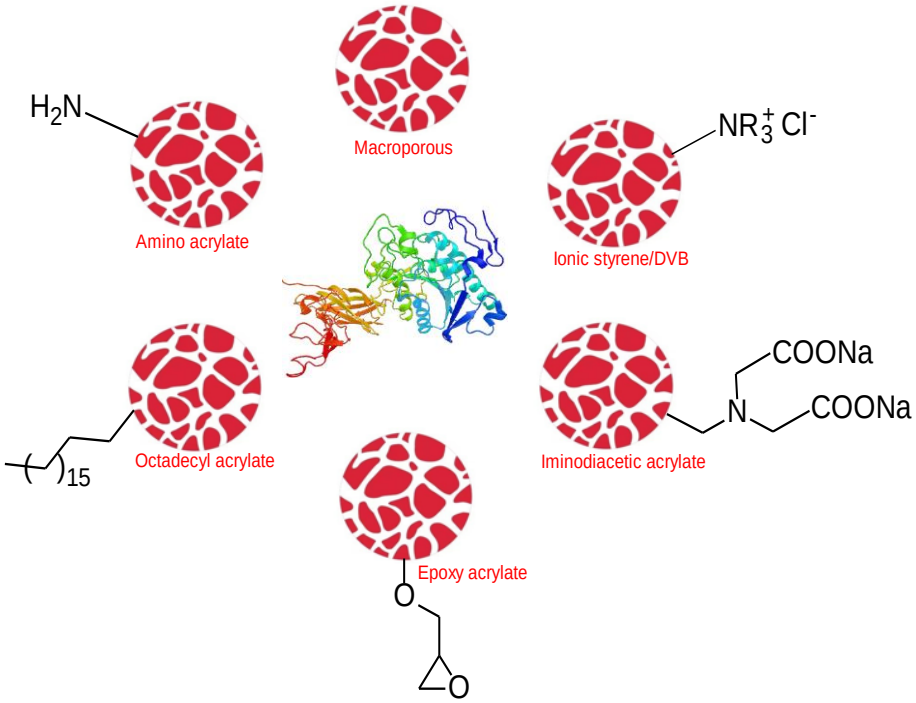
Immobilized enzymes are applied to a variety of processes providing the following benefits:

- Multiple reuse of the biocatalyst
- Easier separation of products
- Flexibility in reactor design
- Ability to operate in aqueous or organic solvents

Seplife® EMC resins are designed for covalent, ionic, affinity and adsorption immobilization.

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

Adsorption is a physical process that exploits interaction between the enzyme and the carrier (polymer). Physical interaction can be very strong and enzymes that have been immobilized like this can be used for many cycles.




References

1.Basso A., Serban S., “Industrial applications of immobilized enzymes—A review”, Molecular Catalysis, 2019, 479, 110607-110627.

2.Sheldon R. A., Basso A., Brady D., “New frontiers in enzyme immobilization: robust biocatalysts for a circular bio-based economy”, https://pubs.rsc.org/en/content/articlelanding/2021/cs/d1cs00015b, Chem. Soc. Rev., 2021, 50, 5850-5862.



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## 1 Covalent Enzyme Immobilization on Epoxy Resins

Epoxy-activated resins are almost ideal matrices to perform easy immobilization of enzymes since they allow single or multipoint covalent binding between enzyme and resin (Figure 1). Immobilization on epoxy resins is more efficient when using high ionic strength buffers.

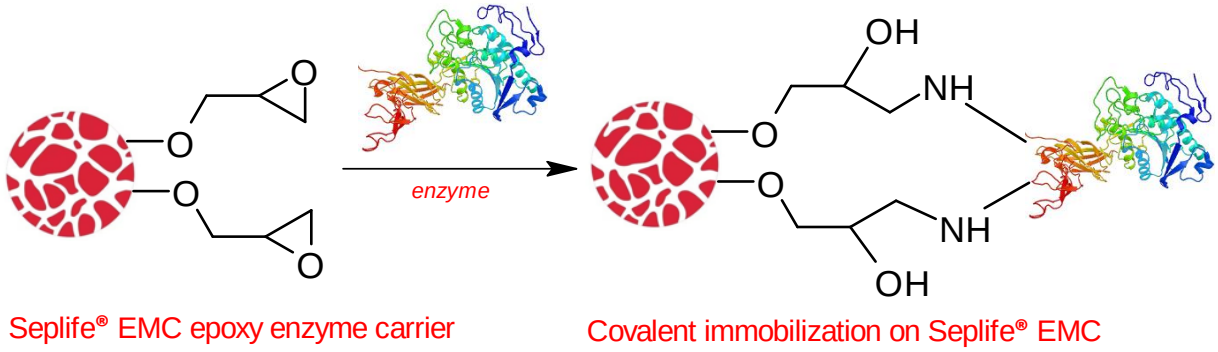


**Figure 1 – Enzyme immobilization schematic on Seplife® EMC Epoxy resins.**

Sunresin Seplife® EMC Epoxy resins include three products that are described in Table 1. These products contain epoxy functional groups and are designed for covalent immobilization of enzymes.

**Table 1 – Seplife® EMC Epoxy resins and their properties.**

| Product | Type | Functional group | Immobilization | Pore diameter (Å) | Particle size (micron)1 | Total moisture (%) |
|---|---|---|---|---|---|---|
| Seplife®EMC7014 | Epoxy acrylate | Epoxy | Covalent | 400-600 | 150-350 | 58-68 |
| Seplife®EMC7025 | Epoxy acrylate | Epoxy | Covalent | 300-500 | 150-350 | 58-68 |
| Seplife® EMC7038 | Epoxy acrylate | Epoxy | Covalent | 400-600 | 125-350 | 60-70 |
| Seplife® EMC7040 | Epoxy acrylate | Epoxy | Covalent | 500-800 | 125-350 | 60-70 |
| Seplife® EMC7042M | Epoxy acrylate | Epoxy | Covalent | 800-1200 | 300-700 | 58-68 |
| Seplife®EMC7032 | Epoxy/butyl acrylate | Epoxy | Covalent | 200-400 | 150-350 | 55-65 |

[1]Customization of particle size is available


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**Table 2 - Mechanical strength and Hydrophilicity of EMC enzyme carriers.**

| Carrier | Matrix | Mechanical strength | Hydrophilicity |
|---|---|---|---|
| Seplife®EMC7014 | Epoxy acrylate | ++ | ++ |
| Seplife®EMC7025 | Epoxy acrylate | +++ | ++ |
| Seplife®EMC7038 | Epoxy acrylate | +++ | ++++ |
| Seplife®EMC7040 | Epoxy acrylate | +++ | +++ |
| Seplife®EMC7042M | Epoxy acrylate | + | +++ |
| Seplife®EMC7032 | Epoxy/butyl acrylate | +++++ | + |

Enzymes covalently immobilized on epoxy functionalized resins are suitable for applications in water or biphasic phase and can be recycled several times until the enzyme is losing activity.


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## Enzyme immobilization protocol using Seplife® EMC Epoxy Resins - guidelines

### Reagents

- Seplife® EMC Epoxy resins
- Native enzyme in liquid or solid form
- Immobilization buffer: Preferably use buffers in which the enzyme is active and stable. Do not use buffers that contain primary amine or thiol groups that could bind to the epoxy functional groups of the resin. Immobilization on epoxy resins is more efficient when using high concentrated buffers (about 1.0M). Select buffers that are stable when used at process temperatures and in large volumes.
- Washing buffer for desorption of non-covalently bound enzyme from the support (0.01 - 0.02M or water). Using a buffer such as Tris after the washing stage can help with blocking remaining active epoxide groups.

### Procedure

**Caution: avoid using magnetic stirring during enzyme immobilization since this can cause damage to the beads.**

**1) Resin equilibration**

Wash the resin with immobilization buffer and filter. A ratio resin/buffer of 1/1 (w/v) is suggested. 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 50-100mg protein per gram of wet resin ($g_{Wet}$). 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 done in later trials as range can vary 1/1 – 1/4 w/v.

**3) Immobilization**

Transfer the immobilization buffer containing the enzyme into the immobilization vessel and add the EMC Epoxy resin. Mix the slurry gently using end to end rotator or overhead stirrer and ensuring the resin is maintained in suspension for 18h and there is no foaming. Stop mixing after 18h and leave without mixing for another 20h. The immobilization can be performed at temperatures between 4 to 30°C depending on enzyme stability. Do not perform immobilizations at high temperatures since this can cause degradation of the epoxy rings and facilitate microbial growth. Immobilization may take longer at low temperatures.

**4) Filtration and washing**

Filter the liquid phase and collect it. Determine the protein content and evaluate the immobilization yield. Wash the resin with the washing buffer. Repeat the process for 2-4 times under gentle mixing using a ration resin: liquid of 1:4 (w/v). An additional washing step using a 0.5M NaCl containing buffer for complete desorption of non-covalently bound proteins is recommended. Remove excess liquid by filtration.

**5) Characterization**

Characterize the immobilized enzyme in terms of moisture content and specific activity.

**6) Storage**

Transfer the immobilized enzyme into a closed container and keep refrigerated (2–8°C).

**Caution: avoid freezing the immobilized enzyme since this may damage the beads.**


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## 2 Covalent Enzyme Immobilization on Seplife® EMC Amino Resins

Another procedure for covalent immobilization of enzymes is based on the use of amino functionalized resins. Amino resins can be pre-activated with glutaraldehyde and then be used in the covalent immobilization of enzymes (Figure 2). Reaction of an aldehyde group with an amino group of the enzymes is fast and forms a *Schiff base*, resulting in a stable single or multipoint covalent binding between enzyme and carrier. An even more stable linkage can be achieved by reduction of the imine double bonds with borohydrides.

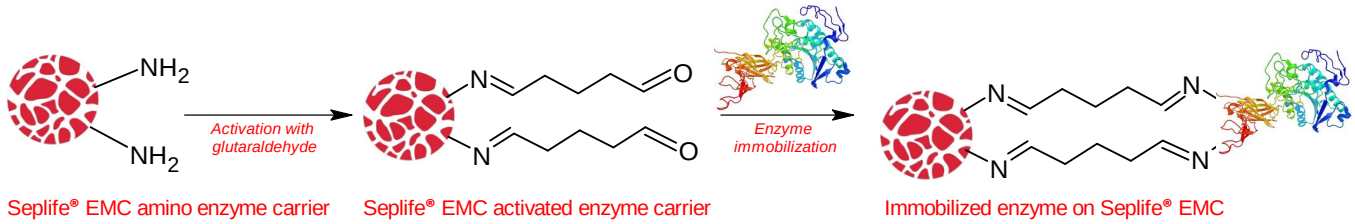


**Figure 2 – Enzyme immobilization schematic on Seplife® EMC Amino resins.**

Sunresin Seplife® EMC Amino resins include the products that are described in Table 3. These products are designed for covalent immobilization of enzymes after pre-activation with glutaraldehyde.

**Table 3 – Seplife® EMC Amino resins and their properties.**

| Carrier | Type | Functional group | Immobilization type | Pore diameter (Å) | Particle size (micron) | Total moisture (%) |
|---|---|---|---|---|---|---|
| EMC7120S | Amino acrylate | Amino (long spacer C6) | Covalent | 200-400 | 100-300 | 50-60 |
| EMC7120M | Amino acrylate | Amino (long spacer C6) | Covalent | 200-400 | 300-700 | 50-60 |
| EMC7225S | Amino acrylate | Amino (short spacer C2) | Covalent | 500-700 | 100-300 | 60-70 |
| EMC7225M | Amino acrylate | Amino (short spacer C2) | Covalent | 500-700 | 300-700 | 60-70 |

Enzymes covalently immobilized on amino functionalized resins are suitable for applications in water or biphasic phase and can be recycled several times until the enzyme is losing activity.


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## Enzyme immobilization protocol on Seplife® EMC Amino Resins - guidelines

### Reagents

- Seplife® EMC Amino resins
- Native enzyme in liquid or solid form
- Immobilization buffer: Preferably buffers in which the enzyme is active and stable. Immobilization on amino resins is efficient when using low concentrated buffers ( 0.01-0.05 M)
- Glutaraldehyde 25% (w/v)

### Procedure

**Caution: avoid using magnetic stirring during enzyme immobilization since this can cause damage to the beads.**

**1) Resin equilibration**

Wash the resin with immobilization buffer and filter. Use preferably a ratio resin/buffer of 1/1 (w/v). Repeat the process for 2-4 times.

**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. Optimization of the glutaraldehyde concentration can be done at later stage.

**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 20-25°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. A change in color of the beads (orange–brown) may occur and is normal. Beads are then ready for the enzyme immobilization step.

**4) Prepare enzyme solution**

Dissolve the native enzyme (liquid or solid) in immobilization buffer. Consider a protein loading of 50-100mg protein per $g_{Wet}$ (gram of wet resin). Protein concentration can be easily determined by using standard protein content assays. Dissolve the enzyme in buffer to obtain a ratio resin/buffer of 1/4.

**5) Immobilization**

Transfer the immobilization buffer into the immobilization vessel and add the EMC pre-activated amino resin obtained in Step 3. Gently mix the slurry for 18 h ensuring the resin is maintained in suspension and there is no foaming. The immobilization can be performed at 4-30°C according 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) Filter and wash**

Filter the liquid phase, collect it and determine the protein content for immobilization yield. Wash with immobilization buffer. Consider additional wash step with 0.5 M NaCl buffer for protein desorption.

**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 (2–8°C).

**Caution: avoid freezing the immobilized enzyme since this may damage the beads.**



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## 3 Enzyme Immobilization by Adsorption on Seplife® EMC macroporous and octadecyl resins

This method for the immobilization of enzymes is based on the physical adsorption of enzyme protein on the surface of water-insoluble carriers (Figure 3). The method is 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.

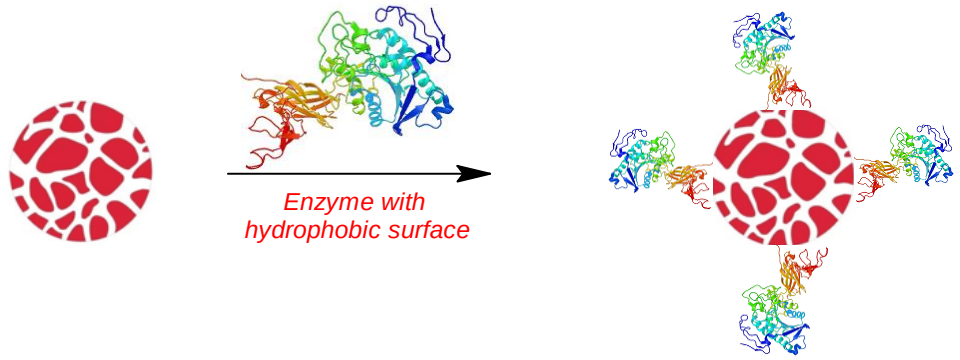


**Figure 3 – Schematic of enzyme immobilization on Seplife® Macroporous styrene resins or Octadecyl resins.**

Sunresin Seplife® EMC resins for adsorption include several products that are described in Table 4. These products are designed for hydrophobic immobilization of enzymes. The final dry immobilized enzyme can be used in organic phase for multiple cycles until the enzyme activity is reduced.

**Table 4 – Seplife® EMC Octadecyl and Macroporous resins and their properties.**

| Carrier | Type | Functional group | Immobilization type | Pore diameter (Å) | Particle size (micron) | Total moisture (%) |
|---|---|---|---|---|---|---|
| EMC7528 | Octadecyl acrylate | Octadecyl (C18) | Adsorption | 200-400 | 400-1000 | 55-65 |
| EMC1020 | Styrene/DVB | None | Adsorption | 500-1000 | 300-900 | 45-55 |
| EMC1040 | Acrylate/DVB | None | Adsorption | 150-300 | 300-700 | 55-65 |


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## Enzyme immobilization protocol on Seplife® EMC Adsorbents - guidelines

### Reagents

- Seplife® EMC Macroporous and Octadecyl resins
- Native enzyme in liquid or solid form
- Buffers for immobilization: Preferably buffers in which the enzyme is active and stable. Immobilization can be performed using low concentrated buffers (about 0.01-0.05 M).

### Procedure

**Caution: avoid using magnetic stirring during enzyme immobilization since this may cause damage to the beads.**

**1) Resin equilibration**

Wash the resin with immobilization buffer and filter. Use preferably a ratio resin/buffer of 1/1. 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 $g_{Wet}$ (gram of wet resin). Protein concentration can be determined using standard protein content assays. Dissolve the enzyme in buffer to obtain a ratio resin/buffer of 1/4 (w/v).

**3) Immobilization**

Transfer the immobilization buffer into the immobilization vessel and add the EMC resin. Gently mix the slurry for 24 h ensuring the resin is maintained in suspension and there is no foaming. The immobilization can be performed at 4-30°C depending on enzyme stability.

**4) Filtration and washing**

Filter the liquid phase and collect it. Determine the protein content and evaluate the immobilization yield. Wash 1-2 times 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 closed container and keep refrigerated (2–8°C).

**Caution: avoid freezing the immobilized enzyme since this may damage the beads.**

**7) Drying**

Adsorbed immobilized enzymes may need to be dried before their use. Drying can be done in a column by bubbling air or Nitrogen up-flow, under vacuum at RT or in an oven with air flow at controlled temperature depending on the enzyme stability.


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## 4 Ionic immobilization of enzymes on Seplife® EMC resins

This method for the immobilization of enzymes is the simples of all and is relying on opposite charges between the enzyme and the resin that will maintain them in contact over repeated cycles. The principle of interaction is presented in the Figure 4 and depends on the attraction and equilibrium between the resin and enzyme charges.

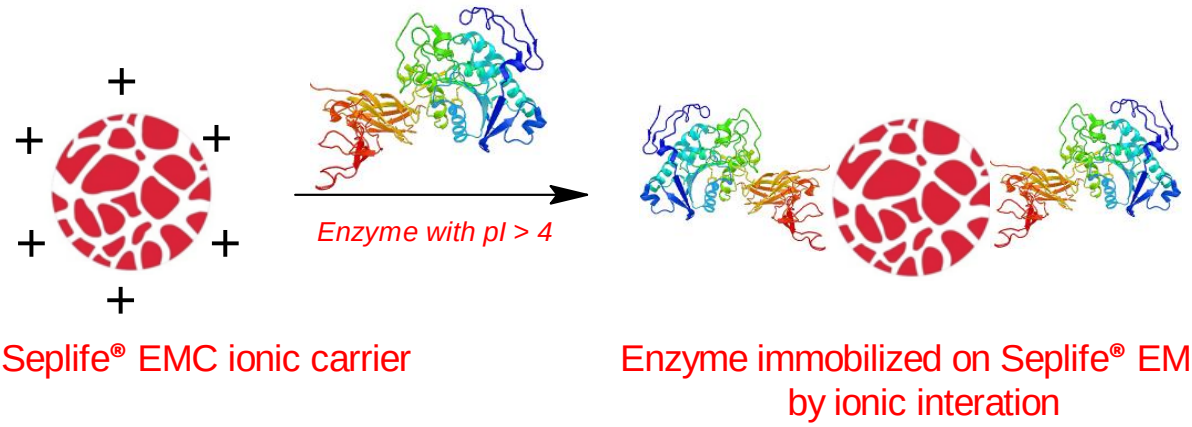


**Figure 4 – Schematic of enzyme immobilization on Seplife® ionic resins.**

Sunresin Seplife® EMC resins for ionic immobilization include the product described in Table 5. This resin has a positive charge at <11 and can form ionic bonds with enzymes negatively charged, when the solution pH is above the isoelectric point of the enzyme.

The final immobilized enzyme can be used in aqueous phase for multiple cycles until the enzyme activity is reduced providing the process is at controlled pH and ionic strength, ensuring the ionic bond between resin and enzyme is stable.

**Table 5 – Seplife® EMC ionic resin and its properties.**

| Carrier | Type | Functional group | Immobilization type | Pore diameter (Å) | Particle size (micron) | Total moisture (%) |
|---|---|---|---|---|---|---|
| EMC7435 | Ionic styrene/DVB | Quaternary amine ($Cl^-$) | Ionic | 500-700 | 315-1250 | 60-70 |

Enzymes such as Invertase, Glucoamylase, Galactosidase, Glucosyl transferase were successfully immobilized by ionic interaction and used in large scale food applications.


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## Enzyme immobilization protocol on Seplife® EMC ionic resin - guidelines

### Reagents

- Seplife® EMC 7435 quaternary amine resins
- Native enzyme in liquid or solid form
- Buffers for immobilization: Preferably buffers in which the enzyme is active, stable and charged negative. Immobilization can be performed using low concentrated buffers (about 0.01-0.05 M). Consider the isoelectric point of the enzyme when choosing the immobilization buffer to ensure the correct enzyme charge.

### Procedure

**Caution: avoid using magnetic stirring during enzyme immobilization since this may cause damage to the beads.**

**1) Resin equilibration**

Wash the resin with immobilization buffer and filter. Use preferably a ratio resin/buffer of 1/1. Repeat the process for 2-4 times. Ensure the pH of the resin in solution is as expected.

**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 $g_{Wet}$ (gram of wet resin). Protein concentration can be determined using standard protein content assays. Dissolve the enzyme in buffer to obtain a ratio resin/buffer of 1/4 (w/v).

**3) Immobilization**

Transfer the immobilization buffer into the immobilization vessel and add the EMC resin. Gently mix the slurry for 24 h ensuring the resin is maintained in suspension and there is no foaming. The immobilization can be performed at 4-30°C depending on enzyme stability. Ensure the pH of the mixture enzyme solution and resin is stable and as expected.

**4) Filtration and washing**

Filter the liquid phase and collect it. Determine the protein content and evaluate the immobilization yield. Wash 1-2 times 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 closed container and keep refrigerated (2–8°C).

**Caution: avoid freezing the immobilized enzyme since this may damage the beads.**

**7) Drying**

If required, ionic immobilized enzymes may be dried before use. Drying can be done in a column by bubbling air or Nitrogen up-flow, under vacuum at RT or in an oven with air flow at controlled temperature depending on the enzyme stability.


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## 5 Enzyme immobilization by affinity on Seplife® Chelex resin

This method for the immobilization of enzymes is based on the affinity interaction between metal ions such as $Ni^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{3+}$ on the resin and the His-tag group on the enzyme.
The principle of interaction is presented in the Figure 5.

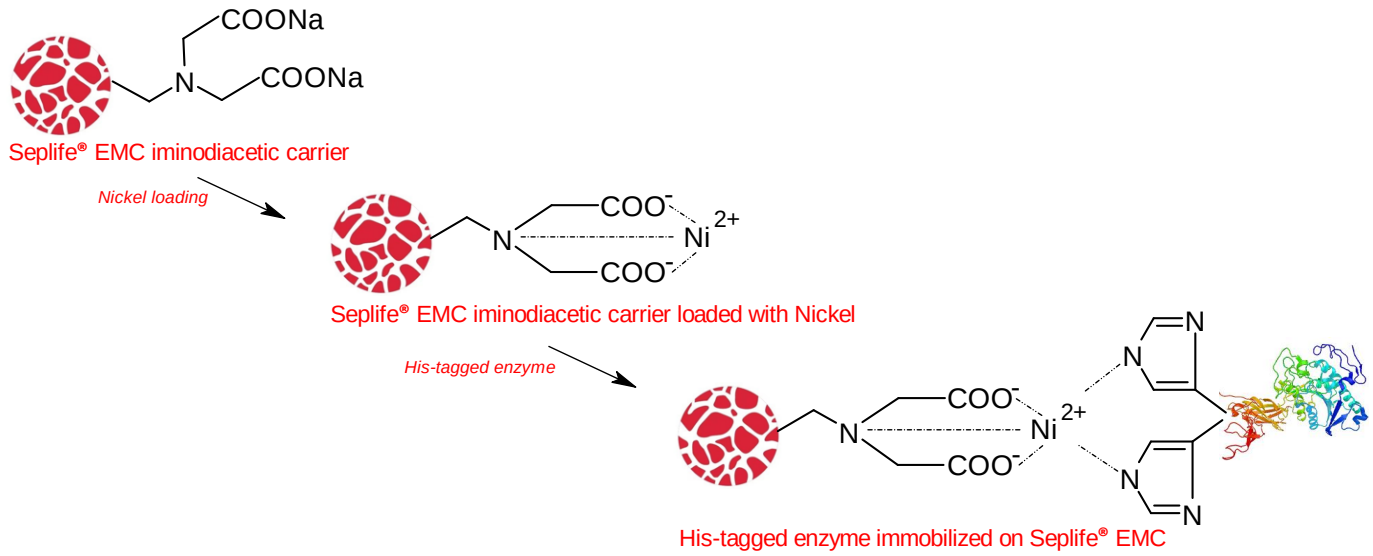


**Figure 5 – Schematic of enzyme immobilization on Seplife® affinity resins.**

Sunresin Seplife® Chelex 7350 resin for affinity immobilization include the product described in Table 6. This resin is functionalized with iminodiacetic groups which can complex metal ions such as $Ni^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{3+}$.
The final immobilized enzyme can be used in aqueous phase for multiple cycles until the enzyme activity is reduced providing the process is controlled and chelating agents such as EDTA, citrates or very low pH are not used.
Sunresin Seplife® Chelex 7350 resin is supplied in $Na^+$ form.

**Table 6 – Seplife® EMC Affinity resin and its properties.**

| Carrier | Type | Functional group | Immobilization type | Pore diameter (Å) | Particle size (micron) | Total moisture (%) |
|---|---|---|---|---|---|---|
| Chelex7350 | Iminodiacetic acrylate | Iminodiacetic ($Na^+$) IDA | Affinity | 800-1000 | 100-250 | 60-70 |

In this case, the use of 0.25-1.0M imidazole will lead to enzyme removal from the resin. This can provide an option for removal of inactive enzyme and reloading of fresh enzyme on the same metal ion loaded resin.


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## Metal loading on Seplife® Chelex 7350 affinity resin - guidelines

### Reagents

- Seplife® Chelex 7350 resin functionalized with iminodiacetic acid
- Metal ion solution such as $Ni^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{3+}$ sulphate or chloride salts can be used and should be fully dissolved in solution.
- Water

### Procedure

**Caution: avoid using magnetic stirring during enzyme immobilization since this may cause damage to the beads.**

1. **Resin equilibration**

   Rinse the resin with water and filter 2-4 times. Remove the liquid. Suspend the Chelex 7350 in $Na^+$ form in water in a ratio 1:1 (w/w).

2. **Preparation of the metal solution and activation of the resin**

   Dissolve the metal salt in water until completely solubilized.
   Transfer the salt solution to the resin slurry targeting a loading of 0.2mmol metal per ml of wet resin. Gently mix the slurry for approx. 3h at RT ensuring the resin is in suspension.

3. **Filtration and washing**

   After the metal activation is completed, remove the supernatant and replace it with water. Wash the resin for 5-7 times with water in a ratio resin: liquid of 1:2 (w/w) by mixing. The supernatant after washing should be clear. If after 5-7 times washing there is still some color in the supernatant, continue washing until the solution is clear. For clear metal solution a conductivity meter can be used to follow the removal of excess metals from the resin.
   Remove all excess liquid by filtration.

4. **Storage**

   Transfer the metal activated resin to a closed container and store at 2-30°C and use for enzyme immobilization by affinity. The metal loaded resin can be stored in these conditions for up to 6 month.


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## Enzyme immobilization protocol on Seplife® Chelex 7350 affinity resin - guidelines

### Reagents

- Seplife® Chelex 7350 resin functionalized with iminodiacetic acid and loaded with metal ions such as $Ni^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{3+}$
- Native enzyme in liquid or solid form
- Buffers for immobilization: Preferably buffers in which the enzyme is active and stable. Immobilization can be performed using low concentrated buffers (about 0.01-0.05 M). Avoid using chelating agents, citrates and high concentrations of imidazole in the enzyme immobilization and washing buffers.

### Procedure

1. **Caution: avoid using magnetic stirring during enzyme immobilization since this may cause damage to the beads.**

2. **Resin equilibration**

   Wash the resin pre-loaded with metal ions such as $Ni^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{3+}$ with immobilization buffer and filter. Use preferably a ratio resin/buffer of 1/1. Repeat the process for 2-4 times.

3. **Preparation of the enzyme solution**

   Dissolve the native enzyme (liquid or solid) in immobilization buffer. Consider a protein loading of 20-50 mg protein per $g_{Wet}$ (gram of wet resin). The protein loading depends on the type of metal on the resin and the type of protein to be immobilized. Protein concentration can be determined using standard protein content assays. Dissolve the enzyme in buffer to obtain a ratio resin/buffer of 1/4 (w/v).

4. **Immobilization**

   Transfer the immobilization buffer into the immobilization vessel and add the Chelex 7350 resin preloaded with metal ions such as $Ni^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{3+}$. Gently mix the slurry for 4-8 h ensuring the resin is maintained in suspension and there is no foaming. The immobilization temperature depends on enzyme stability.

5. **Filtration and washing**

   Filter the liquid phase and collect it. Determine the protein content and evaluate the immobilization yield. Wash 1-2 times with washing buffer (ratio resin/buffer of 1/1 (w/v)).

6. **Characterization**

   Characterize the immobilized enzyme in terms of moisture content and specific activity.

7. **Storage**

   Transfer the immobilized enzyme into a closed container and keep refrigerated (2–8°C).

   **Caution: avoid freezing the immobilized enzyme since this may damage the beads.**


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**References**

1) Metal Affinity Fusion Enzyme Immobilization: Batch Process Showcase for Cascading Alcohol Oxidation and Bayer–Villiger Oxidation in Microaqueous Media, G. Vernet, Y. Ma, S. Serban, A. Basso, N. Zhang, S. Kara, https://doi.org/10.1021/acssuschemeng.4c02236, *ACS Sustainable Chem. Eng.* 2024, 12(29), 10820-10830.

2) Optimization of Metal Affinity Ketoreductase Immobilization for Application in Batch and Flow Processes, A. Basso, M. S. Brown, A. Cruz-Izquierdo, C. A. Martinez, S. Serban, https://pubs.acs.org/doi/10.1021/acs.oprd.1c00483, *Org. Process Res. Dev.*, **2022**, 26, 2075 - 2084.





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