MagReSyn® Carboxyl

Carboxyl-functional magnetic microparticles

Ordering Information0	
Cat. No.	**Quantity**
MR-CBX002	2 ml
MR-CBX005	5 ml
MR-CBX010	2 x 5 ml

This product is for research use only

Table of Contents:

1. Product Description

1.1. Overview

MagReSyn® Carboxyl is a proprietary magnetic polymeric microparticle support designed for covalent attachment of aminated ligands or biological molecules to the polymer microparticle. This may be achieved through an aqueous, carbodiimide-mediated activation process using EDC [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide] and N-hydroxysuccinimide (NHS or Sulfo-NHS) agents in either a single-step coupling reaction or, more commonly, in a two-step reaction where the amine-containing molecule is added subsequent to activation of the carboxyl residues present on the microparticles. The ReSyn technology is differentiated from alternative microparticle technologies in that it comprises a hyper-porous polymer network that allows penetration and binding of biomolecules throughout the volume of the microparticle, leading to a general increase in capacity for the binding of biological molecules. This advance in polymer technology offers an exceptionally high binding capacity for the immobilization of biomolecules.

1.2. Advantages of MagReSyn® Technology

The exceptionally high functional group density of MagReSyn® Carboxyl microparticles provides a high concentration of reactive sites for biomolecule conjugation. The high loading/binding capacity of MagReSyn® allows for miniaturization of experimental protocols by using reduced volumes of highly active functional microparticles and further minimizes the volume of reagents required, allowing application of your immobilized ligands in reduced volumes. The compressibility of the polymer microparticles reduces the interstitial spaces between them during washing and elution procedures, leading to increased efficiencies and recoveries. MagReSyn® microparticles are separated rapidly (<10 s) using a standard magnetic separator, in comparison to leading competitor microparticle technologies which can take up to 4 min to clear. The strong magnetic property of MagReSyn® further minimizes potentially costly loss of sample by preventing accidental discarding/aspiration of the microparticles, resulting in improved experimental reproducibility. The microparticles and recommended buffers are engineered to deliver maximum binding capacity to meet your stringent R&D requirements and enable applications requiring maximum sample loading.00

MagReSyn® Technology Advantages	End-user Benefits
High biological binding capacity of ≥20 mg.ml^{-1} microparticle suspension	Miniaturization of experiments Reduced reagent volumes High density of immobilized ligands
Rapid magnetic separation	Reduced particle carry-over Improved experimental reproducibility Rapid protocols
Resistant to oxidation (rust)	Reduced sample contamination Longer shelf life
Multipoint covalent coupling	Stable coupling Reduced leaching of ligand Enhanced stability of ligand

1.3. Product Information

Product Specifications	
Description	Iron oxide-containing magnetic polymer microparticles
Application	Immobilization of proteins, peptides, enzymes, antibodies and other ligands
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Functional group	Carboxylic acid (COOH)
Binding capacity	≥20 mg.ml^{-1} (BSA)
Particle Size	~5–10 µm
Formulation	2%: 20 mg.ml^{-1} suspension in 20% ethanol
Stability	pH 3.5–10; 4–60°C
Storage	Store at 4–8°C until expiry date on label **DO NOT FREEZE**

1.4. Additional Equipment and Materials

Magnetic separator, Vortex mixer, Buffers and solutions, End-over-end mixer (optional).

2. Binding Procedure

2.1. Sample Preparation

Important considerations for efficient ligand immobilization are the ionic strength and pH of the coupling buffer. The coupling buffer should be free of primary amine-containing compounds (e.g. Tris). Please read the instruction guide carefully to ensure you are using suitable coupling buffers. If the sample to be coupled contains contaminants such as salts, sugars, stabilizers or chemical compounds that may interfere with the coupling reaction, these components should be removed from the sample by a suitable technique such as desalting, ultrafiltration, or similar. The coupling efficiency to NHS-EDC-activated carboxyl microparticles is ligand dependent and may require optimization to achieve desired results.

NOTE: All reagents should be freshly prepared and of analytical grade to ensure optimal performance. The procedures, methods and buffer solutions and ligands described below serve as an example and are not intended to be limiting. MagReSyn® Carboxyl is compatible with a range of different buffers for ligand immobilization. Capacity is ligand dependent and should be optimized to ensure desired results.

2.2. MagReSyn® Carboxyl Equilibration

MagReSyn® Carboxyl is supplied as a 20 mg.ml^{-1} suspension in 20% ethanol. The shipping solution needs to be removed and the microparticles equilibrated in binding buffer before use. The recommended protocol can be scaled up or down to suit your requirements - the current protocol is estimated for binding ~1 mg of protein.

1) Resuspend MagReSyn® Carboxyl thoroughly by vortex mixing for 3 s to ensure a homogenous suspension.
2) Transfer 50 µl (1 mg) MagReSyn® Carboxyl to a new tube. We recommend using a low-binding tube such as Eppendorf® Protein LoBind.
3) Place the tube on the magnetic separator and allow the microparticles to clear.
4) Remove the shipping solution by aspiration with a pipette and discard.
5) Wash/equilibrate the microparticles in 200 µl of compatible buffer selected for ligand conjugation (e.g. activation buffer, 0.1 M MES section **2.3**), allow 1 min for equilibration.
6) Place the tube on the magnetic separator and allow the microparticles to clear. Invert the magnet twice with the tube in place to collect any microparticles remaining in the cap.
7) Remove the binding buffer by aspiration with a pipette and repeat steps 5 and 6 twice for a total of three washes.
8) After removal of the buffer from step 6, MagReSyn® Carboxyl is ready to be functionalized for ligand binding.

2.3. Activation of MagReSyn® Carboxyl with Carbodiimide and N-hydroxysuccinimide, NHS

1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) is a water-soluble coupling agent that can be used to activate carboxyl groups present on the microparticles to provide an intermediate ester capable of reacting with the primary amines present on e.g. proteins:

1) Prepare sufficient activation solution for your immobilization requirements. Activation solution contains: 20 mM EDC and 50 mM NHS in a suitable buffer, e.g. 0.1 M MES, 0.5 M NaCl, pH 6.0. *Example: Dissolve 28.8 mg of NHS and 19.1 mg of EDC in 5 ml of buffer. Ensure the EDC and NHS are completely dissolved before use.*
2) Add 0.5 ml activation solution to the 1 mg equilibrated microparticles prepared according to **2.2**.
3) Agitate microparticle suspension for 15 min at room temperature. We recommend slow end-over-end mixing.
4) Place the tube on the magnetic separator and allow the microparticles to clear. Aspirate the supernatant with a pipette and discard.
5) Wash the microparticles three times with 1 ml of immobilization-compatible buffer, e.g. PBS, pH 7.0. Allow 10 s for equilibration between each wash procedure.

2.4. Protein Coupling Procedure

Note: If your protein content for immobilization is low, particle aggregation may occur. If you do not have sufficient protein for coupling to the beads, a carrier protein such as BSA or casein may be mixed with the protein of interest to provide sufficient content to avoid aggregation.

1) Prepare protein to be coupled in a suitable coupling buffer (free of primary amines, e.g. PBS, pH 7.0, and add to the microparticle suspension.
2) Agitate using end-over-end mixer or gentle vortexing for 2–24 h at room temperature or at 4˚C for temperature-sensitive ligands.
3) Place the tube on the magnetic separator and allow the microparticles to clear.
4) Remove the unbound fraction by pipette aspiration and discard or keep for subsequent determination of coupling efficiency.
5) Wash twice with 200 µl coupling buffer only (equilibration for 1 min between washes). Aspirate and discard the wash fractions using a magnetic separator.
6) Add 500 µl of a suitable buffer comprising excess amine-containing blocking agent (e.g. 200 mM ethanolamine or aspartic acid in PBS, pH 7.0) and incubate for 3 h at room temperature (or 12+ hours at 4ºC) to quench any remaining amine-reactive residues on the microparticles.
7) Apply magnetic separator and aspirate and discard excess quenching agent.
8) **Optional**: Wash three times with 500 µl suitable buffer solution containing 0.5–1 M NaCl to remove possible non-covalently bound proteins and non-reactants. Apply a magnetic separator to recover microparticles and aspirate wash solutions.
9) Wash/equilibrate the microparticles containing your immobilized ligand in a suitable volume of buffer for your further applications or storage. The microparticles are now ready for your downstream experimentation.
10) Immobilized biologicals may be stored with suitable preservatives (e.g. sodium phosphate buffer containing 0.05% sodium azide) at 4˚C. **DO NOT FREEZE** the microparticles.

3. Storage

MagReSyn® Carboxyl is supplied as a suspension of 20 $mg.ml^{-1}$ in 20% ethanol and should be stored at 2–8ºC until the expiry date on the label. **DO NOT FREEZE**. Improper storage, drying of microparticles, bacterial contamination, or centrifugal recovery may result in irreversible loss of capacity/performance. Resuspend well by vortex mixing before use.

4. General Information & Disclaimers

Contact us at info@resynbio.com for larger microparticle quantities or customized microparticle solutions for your application. Visit our website (***www.resynbio.com***) for more information on the ReSyn technology platform and other available products. This product is for research purposes only. The product contains 20% ethanol as a preservative. The product is meant for single use only and not recommended for reuse. When working with laboratory reagents, always wear suitable personal protective equipment including a lab coat, disposable gloves, and safety glasses. For further safety information please consult our Material Safety Data Sheet (**MSDS**), which is available for download at www.resynbio.com. Storage solutions, chemical reagents, buffers and biologicals should be suitably disposed of with adherence to your local waste-disposal legislation. MagReSyn® is a registered trademark of ReSyn Biosciences (Pty) Ltd, South Africa. ReSyn Biosciences (Pty) Ltd, distributors, agents or representatives, will not be held responsible for patent violations or infringements occurring as a result of using our products. In no event shall ReSyn Biosciences (Pty) Ltd be liable for any direct, indirect, punitive, incidental or consequential damage to property or life, whatsoever arising out of or connected with the use or misuse of its products. Please consult our website for further general disclaimers.

5. Troubleshooting Guide

Identified Problem	Possible Cause	Suggested Remedy
Ligands do not bind to the microparticles as expected	Activation solution unstable	Use freshly prepared activation solution
	Incorrect activation and binding pH	Ensure that the activation pH is between 5-6.5 Check calibration of pH meter
	Protein of interest degraded	Add protease inhibitors or prepare fresh protein solution
	Interfering compounds in sample or solutions prevent binding	Ensure buffers are primary amine-free, e.g. no Tris or glycine buffers are to be used. Desalt ligand/biological according to best current practice.
	Insufficient microparticle quantity	Increase quantity of MagReSyn® Carboxyl microparticles
	Protein/ligand content too low	Apply a more concentrated ligand solution. Add BSA or other carrier proteins, e.g. Casein or BGG to increase the total protein concentration to between 2–10 $mg.ml^{-1}$

Please contact us via e-mail at info@resynbio.com should your specific problem not be addressed in our troubleshooting guide.

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