MagReSyn® Amine

Primary amine functional magnetic microparticles

Ordering Information	
Cat. No.	**Quantity**
MR-AMN002	2 ml
MR-AMN005	5 ml
MR-AMN010	2 x 5 ml

This product is for research use only

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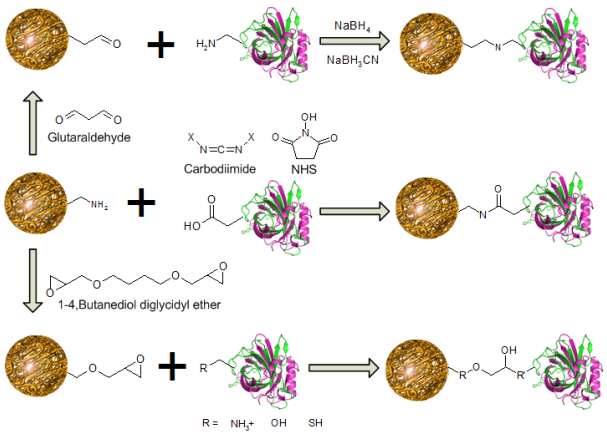
1. Product Description

1.1. Overview

MagReSyn® Amine is a proprietary magnetic polymeric microparticle support designed for the covalent attachment of biological ligands through chemical activation of the microparticles (or the biologicals) with a variety of chemical coupling agents. The current protocol provides a protocol for activation of the amine beads to epoxide for the subsequent coupling of protein for application in example immunoprecipitation. Activation takes place by the addition of a bifunctional crosslinkers 1,4-butanediol diglycidyl ether.



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® Amine 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 microparticles 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.

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 magnetophoretic mobility	Reduced particle carry-over Improved experimental reproducibility Rapid protocols
Resistant to oxidation (rust)	Reduced sample contamination Improved shelf-life
Multipoint covalent attachment	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	Primary amine
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^{o}C
Storage	Store at 4–8^{o}C until expiry date on label **DO NOT FREEZE**

1.4. Reagents supplied

- MagReSyn® Amine (20 mg.ml^{-1} in 20% ethanol)

Additional Equipment and Materials

- Magnetic separator, Vortex mixer, Buffers and solutions, end-over-end mixer (or similar).
- Activation Solution (5.16 M 1,4-butanediol diglycidyl ether)
- Blocking Reagent 1 (250 mM Ethanolamine pH 8.5)
- Blocking Reagent 2 (250 mM Aspartic acid pH 8.5)

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 can 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 is ligand dependent and may require optimization to achieve desired results. EDC/NHS-activated proteins should be desalted before addition to MagReSyn® Amine since these by-products may interfere with coupling.

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

2.2. MagReSyn® Amine Equilibration

MagReSyn® Amine 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.

1) Resuspend MagReSyn® Amine thoroughly by vortex mixing for 3 s to ensure a homogenous suspension.
2) Transfer 50 µl (1 mg) MagReSyn® Amine to a new tube. We recommend using a low-binding tube such as Eppendorf® Protein LoBind.

Patent numbers WO2009057049; IN267234; US9574054; ES2595104; EP2205662; DK2205662; CN101874061; CA2703478; JP五四四二六二四

3) Place the tube on the magnetic separator and allow the microparticles to clear.
4) Remove and discard the shipping solution by aspiration with a pipette.
5) Wash/equilibrate the microparticles in 200 µl of ultrapure water.
6) Place the tube on the magnetic separator to allow the microparticles to clear and aspirate supernatant with a pipette.
7) Repeat steps 5 and 6 for a further two washes.
8) After removal of the water, MagReSyn® Amine is ready for functionalization for activation.

2.2. Activation of MagReSyn® Amine to Epoxide

We provide an below a protocol suitable for epoxide activation for subsequent biological ligand coupling. 1,4-Butanediol diglycidyl ether is a homo-bifunctional coupling agent reactive towards thiol, amine, and hydroxyl groups and can be used to activate the surface of the amine-functional microparticles to provide epoxide groups.

NOTE: The protocol provided below serves as a guideline and is not intended to be limiting. Volumes may be scaled appropriately to optimize coupling efficiency.

1) The activation solution consists is prepared by preparing a solution of 1.5 M 1,4-butanediol diglycidyl ether in water. Prepare a suitable volume of activation solution per reaction e.g. for a 50 µl sample add we recommend 100 µl of activation solution (i.e. 29 µl of high purity 1,4-butanediol diglycidyl ether activation solution to 71 µl ultrapure water).
2) Add the activation solution to the washed/equilibrated microparticles from **2.1** and resuspend well by vortex for 3 s.
3) Agitate for 48 h at room temperature (e.g. end-over-end or gentle vortexing). *Ensure that the microparticles stay in suspension for the entire activation process to ensure efficient microparticle interaction with the activation solution.*
4) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the supernatant with a pipette.
5) Wash twice with 200 µl of ultrapure water followed by a further two washes with suitable amine-free binding/coupling buffer (e.g. the solution that will be used for ligand immobilization).

2.3 Ligand Coupling Procedure

1) Prepare ligand to be coupled in a suitable coupling buffer (free of primary amines) .
2) Add the ligand/protein of interest to be immobilized to the activated microparticle suspension from **2.2** at the required concentration in an appropriate coupling buffer
3) Agitate using an end-over-end mixer or gentle vortexing for 24–48 h at room temperature or at 4˚C for temperature-sensitive ligands. *Ensure that the microparticles remain suspended for the entire coupling process to ensure sufficient interaction between the microparticles and sample.*
4) Place the tube on the magnetic separator and allow the microparticles to clear.
5) Remove the unbound fraction by pipette aspiration and discard, or keep for subsequent determination of coupling efficiency.
6) Wash twice with 200 µl coupling buffer only (equilibrate for 1 min between washes). Aspirate and discard the wash fractions using a magnetic separator.
7) Add 100 µl of a suitable buffer containing excess amine-containing blocking agent e.g. 250 mM ethanolamine pH 8.5 (Blocking Solution 1) or 250 mM aspartic acid pH 8.5 (Blocking Solution 2) and incubate for 16-24 h at room temperature (or 4ºC for temperature-sensitive ligands) to quench any remaining epoxide residues on the microparticles.
8) Apply magnetic separator and aspirate and discard excess blocking agent.
9) Wash the microparticles three times with 500 µl suitable buffer solution containing 0.5–1 M NaCl (e.g. PBS or Tris) to remove possible non-covalently bound ligand and blocking reagents. Use a magnetic separator to recover microparticles and aspirate wash solutions.
10) Wash/equilibrate the microparticles containing your immobilized ligand in a suitable volume of buffer for storage or further applications. The microparticles are now ready for your downstream experimentation.
11) Immobilized ligands may be stored with suitable preservatives (e.g. sodium phosphate buffer containing 0.05% sodium azide) at 4˚C. **DO NOT FREEZE** microparticles.

3. Recommended Storage

MagReSyn® Amine 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 expired	Use freshly prepared activation solution
	Ligand of interest degraded	Validate ligand or use fresh ligand
	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® Amine particles
	Protein/ligand content too low	Apply a more concentrated ligand solution.
	Inefficient mixing	Ensure that the microparticles are always thoroughly mixed during activation and ligand coupling steps.

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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