



ReSyn Biosciences

## MagReSyn® Amine

Primary amine functional magnetic microparticles

Ordering Information	
Cat. No.	Quantity
MR-AMN002	2 ml
MR-AMN005	5 ml
MR-AMN10	2 x 5 ml

This product is for research use only

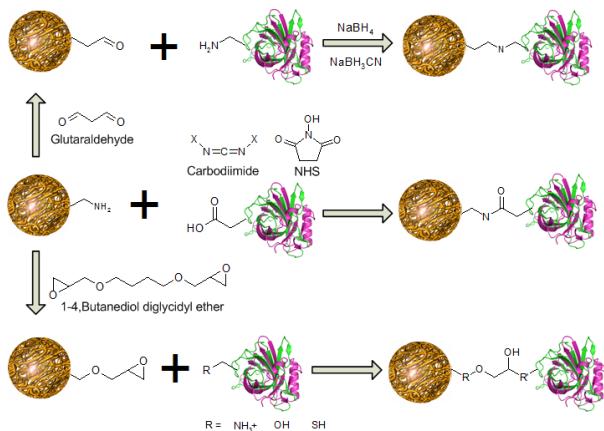
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#### 1. Product Description

##### 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 most frequently used activation reagents include N-hydroxysuccinimide (NHS or Sulfo-NHS) and carbodiimide derivatives (such as 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride), as well as bifunctional crosslinkers such as glutaraldehyde or 1,4-butanediol diglycidyl ether.



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 $\geq 20 \text{ mg.ml}^{-1}$ 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	$\geq 20 \text{ mg.ml}^{-1}$ (BSA)
Particle Size	$\sim 5\text{--}10 \mu\text{m}$
Formulation	2%: 20 mg.ml <sup>-1</sup> suspension in 20% ethanol
Stability	pH 3.5–10; 4–60°C
Storage	Store at 4–8°C until expiry date on label <b>DO NOT FREEZE</b>

#### 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 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 desalts 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<sup>-1</sup> 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® 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.
- 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 activation/immobilization buffer selected for ligand conjugation (section 2.2).
- 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 buffer, MagReSyn® Amine is ready for functionalization for ligand binding.

## 2.2. Activation of MagReSyn® Amine: Example

We provide an example of a suitable glutaraldehyde activation protocol for the coupling of an aminated ligand. Glutaraldehyde is a homo-bifunctional amine-reactive coupling agent that can be used to activate the surface of the amine-functional microparticles and provide aldehyde groups for the subsequent coupling of amine containing ligands.

**NOTES:** The protocol may be adapted for coupling using alternative homo- or hetero-functional coupling agents. Glutaraldehyde and other bifunctional coupling reagents are often hazardous and/or toxic and care should be taken in their use.

- 1) Prepare 200 µl of an activation solution consisting of 5% glutaraldehyde in ultrapure water (or immobilization-compatible buffer such as 50 mM triethanolamine, pH 8.0) *NOTE: the amine-aldehyde reaction is favored at alkaline pH and activation/immobilization solutions should have a pH ≥8.0*
- 2) Add the glutaraldehyde solution to the washed/equilibrated microparticles from 2.1 and resuspend well by vortexing for 3 s.
- 3) Agitate for 3 h at room temperature (e.g. end-over-end or gentle vortexing).
- 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.
- 6) Add the ligand/protein of interest to be immobilized to the microparticle suspension at a concentration of 2–10 mg·ml<sup>-1</sup> in an appropriate coupling buffer.

## 2.3 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. triethanolamine, pH 8.0) and add to the microparticle suspension.
- 2) Agitate using an 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 (equilibrate for 1 min between washes). Aspirate and discard the wash fractions using a magnetic separator.
- 6) **Optional:** The coupling of a primary amine to an aldehyde results in Schiff-base bond formation. Stabilization of this bond can be achieved using sodium borohydride or sodium cyanoborohydride. Please consult relevant literature for suitable protocols.
- 7) Add 500 µl of a suitable buffer containing excess amine-containing blocking agent (e.g. 200 mM ethanolamine or aspartic acid in PBS, pH 8.0) and incubate for 3 h at room temperature (or 12+ hours at 4°C) to quench any remaining amine-reactive residues on the microparticles.
- 8) Apply magnetic separator and aspirate and discard excess quenching agent.

- 9) **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. 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 biologicals 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<sup>-1</sup> in 20% ethanol and should be stored at 2–8°C 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](mailto:info@resynbio.com) for larger microparticle quantities or customized microparticle solutions for your application. Visit our website ([www.resynbio.com](http://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](http://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	Glutaraldehyde solution expired	Use freshly prepared 5–10% glutaraldehyde
	Incorrect binding pH	Ensure pH is >8.0 Check calibration of pH meter
	Protein of interest degraded	Add protease inhibitors to extract
	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. Add BSA or other carrier proteins e.g. Casein or BGG to increase the total protein concentration to 2–10 mg·ml <sup>-1</sup>

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