MagReSyn® Streptavidin MS

Affinity binding/capture of biotinylated biomolecules

Ordering Information	
Cat. No.	**Quantity**
MR-STP002	2 ml
MR-STP005	5 ml
MR-STP010	2 x 5 ml

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® Streptavidin for **Mass Spectrometry (MS)** is a proprietary magnetic polymeric microparticle support that provides a simple and convenient method for the isolation or immobilization of biotinylated biomolecules including proteins and nucleic acids. The ReSyn microparticle technology is differentiated from conventional solid or cracked bead technologies in that it is a hyper-porous polymer network, which allows penetration and binding of biomolecules throughout the volume of the microparticle. This facilitates exceptional streptavidin binding capacity that in turn translates to high capacity for the binding of target biotinylated biomolecules. The product consists of recombinant streptavidin (55 kDa) covalently linked to magnetic microparticles. The high functional group density used for immobilization enables maximum biomolecule loading, increased stability and reduced potential for streptavidin leaching. MagReSyn® Streptavidin MS has the added benefit of being chemically treated to modify lysine and arginine residues, which reduces sample contamination of streptavidin peptides resulting from proteolytic digestion using LysC and trypsin. This product is specifically designed for applications requiring on-bead digestion prior to MS analysis such as BioID. Please consult our Method Library (resynbio.com/method-library/) for the latest advances in applications such as BioID, and MS compatible conditions for using this product.

1.2. Advantages of MagReSyn® Technology

The exceptional biological 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 recovery of valuable biologicals in reduced volumes. In addition, the compressibility of the microparticles reduces the interstitial spaces between the microparticles 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 alternative microparticle technologies that may 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 target proteins of exceptional purity to meet your stringent R&D requirements.

MagReSyn® Technology Advantages	End-user Benefits
High specificity for biotinylated biomolecules	High purity of target proteins Low non-specific interactions
Exceptionally high binding capacity	Miniaturization of experiments Reduced reagent volumes Increased sample concentration Improved recovery of valuable biologicals
Rapid magnetic separation	Reduced particle carry-over Improved experimental reproducibility Rapid protocols
Multipoint covalent attachment of streptavidin, chemical modification of streptavidin lysine and arginine residues	Improved streptavidin stability Reduced streptavidin leaching Possibility of working under non-standard denaturing conditions Reduced contamination from endogenous streptavidin peptides post on-bead digestion
Resistant to oxidation (rust)	Reduced sample contamination Longer shelf life

1.3. Product Information

Product Specifications	
Description	Iron oxide-containing magnetic polymer microparticles
Application	Isolation and purification of biotinylated biomolecules
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Functional group	Streptavidin (55 kDa)
Binding capacity	≥2,500 pmoles.mg^{-1} biotinylated oligonucleotide (24 mer), ≥250 µg.mg^{-1} biotinylated IgG
Particle Size	~5–10 µM
Formulation	1%: 10 mg.ml^{-1} in 80 mM Phosphate, pH 7.5, 150 mM NaCl, 1.5 mM EDTA, 0.05% Tween® 20, 0.02% sodium azide (NaN_3)
Stability	pH 3–10; 4–60ºC
Storage	Store at 4–8ºC until expiry date on label **DO NOT FREEZE**

1.4. Additional Equipment and Materials

Magnetic separator, Vortex mixer, Pipettes Buffers and solutions

RIPA Buffer: 50mM Tris pH 7.5, 150mM NaCl, 1% NP-40, 0.2% SDS, Protease inhibitor cocktail
Wash Buffer 1: RIPA Buffer
Wash Buffer 2: 3M Urea in 50mM TEAB pH 8.5
Wash Buffer 3: 6M Urea in 50mM TEAB pH 8.5
Reduction & Alkylation Buffer: 50mM TEAB pH 8.5, containing 5mM TCEP and 10mM CAA
Digestion Buffer: 50mM TEAB pH 8.5

2. Binding and Elution Procedure

Factors that may affect the attachment of biotinylated biomolecules include buffer composition and pH and the presence of contaminants/interfering compounds. Although both large and small molecules can be immobilized on the MagReSyn® Streptavidin MS microparticles, the size of the biotinylated molecule may affect the overall binding capacity. The quantity of microparticles required may therefore require optimization for your application. Best results for downstream applications may be achieved with microparticles saturated with biotinylated molecules. The efficiency of biotinylated molecule binding can be determined by comparing the molecule concentration in solution before and after coupling reactions. MagReSyn® Streptavidin is compatible with various commonly used buffers, including Tris, Phosphate and SSC (sodium saline citrate).

NOTE: All reagents should be freshly prepared and of analytical grade to ensure optimal performance. The procedures, methods and buffer solutions described below serve as an example and are not intended to be limiting. MagReSyn® Streptavidin MS is compatible with a range of different buffers commonly used for capturing and/or immobilizing biotinylated molecules. Achievable purity and yield are ligand dependent and experimental conditions should be optimized to ensure desired results.

2.1. MagReSyn® Streptavidin Equilibration

MagReSyn® Streptavidin MS is supplied as a 10 mg.ml^{-1} suspension (80 mM Phosphate, pH 7.5, 150 mM NaCl, 1.5 mM EDTA, 0.05% Tween® 20, 0.02% sodium azide (NaN_3)). The shipping solution needs to be removed and the microparticles equilibrated in binding buffer prior to use. Equilibrate aliquots of MagReSyn® Streptavidin for your requirements as outlined in the protocol below.

NOTE: A minimum volume of 10 µl microparticle suspension is required per reaction to ensure a suitable pellet size for the aspiration of buffers.

1) Resuspend MagReSyn® Streptavidin MS thoroughly by vortex mixing or inversion to ensure a homogenous suspension.
2) Transfer at least 10 µl MagReSyn® Streptavidin MS to a new tube.
3) Place the tube on the magnetic separator and allow the microparticles to clear.
4) Remove the shipping solution by aspiration with a pipette.
5) Wash/equilibrate the microparticles in 500µl of **RIPA buffer**.
6) Place the tube on the magnetic separator and allow the microparticles to clear.
7) Remove the binding buffer by aspiration with a pipette and repeat steps 5 and 6 twice for a total of three washes.
8) When your sample is ready, remove the supernatant and apply the your sample to the equilibrated beads.

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

2.2. Cell Lysis and binding

1) Lyse cells at 4°C in 500µl RIPA buffer (50mM Tris pH 7.5, 150mM NaCl, 1% NP-40, 0.2% SDS, Protease inhibitor cocktail).
2) Add cell lysate to the pre-equilibrated beads from 2.1 and bind for 24 hours at 4°C with side-to-side mixing, sufficient to keep beads in suspension
3) Place the tube on the magnetic separator and allow the microparticles to clear, and remove the supernatant (unbound fraction).

2.3. Washing

1) Add 500µl of ***Wash buffer 1*** (RIPA).
2) Mix the tubes from side to side for 10 minutes.
3) Place the tube on the magnetic separator and allow the microparticles to clear, remove the supernatant and discard.
4) Repeat steps 1 to 3 a further 2 times for a total of 3 washes.
5) Add 500µl of ***Wash buffer 2*** containing 3M Urea in 50mM TEAB pH 8.5
6) Mix the tubes from side to side for 10 minutes.
7) Place the tube on the magnetic separator and allow the microparticles to clear, remove the supernatant and discard.
8) Resuspend the beads in 500µl of ***Wash buffer 3*** containing 6M Urea in 50mM TEAB pH 8.5
9) Mix the tubes from side to side for 10 minutes.
10) Place the tube on the magnetic separator and allow the microparticles to clear, remove the supernatant and discard.
11) Resuspend the beads in 500µl of 50mM TEAB pH 8.5
12) Mix the tubes from side to side for 10 minutes.
13) Place the tube on the magnetic separator and allow the microparticles to clear, remove the supernatant and discard.

2.4. On-bead Reduction, Alkylation, & Digestion for MS analysis

1) Resuspend the beads in 200µl of ***Reduction & Alkylation Buffer*** (50mM TEAB pH 8.5, containing 5mM TCEP and 10mM CAA)
2) Mix the tubes from side to side for 30 to 60 minutes.
3) Place the tube on the magnetic separator and allow the microparticles to clear, remove the supernatant and discard.
4) Resuspend the beads in 200µl of 50mM TEAB pH 8.5, containing 500ng of LysC/Trypsin mix per 1mg of MagReSyn® Streptavidin MS (Promega Cat# V5071 or similar).
5) Mix the tubes from side to side at 37°C for 4 to 18 hours
6) Quench the proteolytic digestion by the additions of formic acid to a final concentration of 1%.
7) Place the tube on the magnetic separator and allow the microparticles to clear.
8) Remove the supernatant containing digested peptides and proceed to your preferred de-salting technique prior to LC MS analysis.

NOTE: Proximity biotinylation is an emerging application of Streptavidin beads, and although this protocol can be used as a guideline, we recommend you consult with the latest publications in this field. Applications using our beads can be accessed via the method library available at https://resynbio.com/method-library/

3. Recommended Storage

MagReSyn® Streptavidin MS is supplied as a 10 $mg.ml^{-1}$ suspension of microparticles in 80 mM Phosphate, pH 7.5, 150 mM NaCl, 1.5 mM EDTA, 0.05% Tween® 20, 0.02% sodium azide (NaN_3) and should be stored at 2–8°C. **DO NOT FREEZE**. Improper storage, drying of microparticles, bacterial contamination, or centrifugal recovery may result in irreversible loss of capacity. Resuspend well by vortex mixing before use.

4. Reagent Compatibility

Please consult the most recent literature to assess the compatibility of your buffers with MagReSyn® Streptavidin MS. We recommend you use the method-library as the preferred resource, since these buffers would have been validated with our beads.

5. 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 sodium azide 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.

6. Troubleshooting Guide

Identified Problem	Possible Cause	Suggested Remedy
Biotinylated biomolecules do not bind to the microparticles as expected	Incorrect binding pH	Adjust pH of binding buffer to between pH 7.5–8.5
	Interfering compounds in sample prevent binding	Desalt or dialyze sample into recommended binding buffer to remove media components or other contaminants
	Insufficient microparticle quantity	Increase amount of MagReSyn® Streptavidin microparticles
	Biomolecule content too low	Increase volume of sample being applied to the beads
	Inefficient biotinylation of target molecule	Refer to the troubleshooting guide of the supplier of your biotin-labelling kit or revisit the literature.
Non-specific binding of non-biotinylated molecules to the microparticles	Non-specificity due to ionic or electrostatic forces	Consult the recent literature to assess the compatibility of your buffers for proximity biotinylation.
	Insufficient washing	Increase number or volume of wash steps. Carefully aspirate excess remaining wash buffer from the microparticles to avoid carry-over.

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