

MagReSyn® Streptavidin

Affinity binding/capture of biotinylated biomolecules

Ordering Information		
Cat. No.	Quantity	
MR-STV002	2 ml	
MR-STV005	5 ml	
MR-STV010	2 x 5 ml	

This product is for research use only

Table of Contents:

- 1. Product Description
- 2. Binding and Elution Procedure
- 3. Recommended Storage
- 4. Reagent Compatibility
- 5. General Information & Disclaimers
- 6. Troubleshooting Guide

1. Product Description

1.1. Overview

MagReSyn® Streptavidin 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 provides an unsurpassed capacity for biotinylated oligonucleotides and proteins. Applications for this product include the isolation of biotinylated nucleic acids and proteins, the isolation of DNA/RNA-binding proteins, cell isolation and immunoassays. A four-fold higher capacity product (MagReSyn® Streptavidin MAX) is also available as a custom preparation, please enquire.

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	High purity of target proteins (≥97%)
biotinylated biomolecules	Reduces additional isolation steps
	Low non-specific interactions
Exceptionally high	Miniaturization of experiments
biological binding capacity	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	Improved streptavidin stability
attachment of streptavidin	Reduced streptavidin leaching
	Possibility of working under non-standard
	denaturing conditions
Resistant to oxidation	Reduced sample contamination
(rust)	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	Recombinant streptavidin (55 kDa)		
group			
Binding capacity	≥3,000 pmoles.mg ⁻¹ biotinylated oligonucleotide		
	(24 mer), ≥300 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; 4–60°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

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 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 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.

NOTE ON MS COMPATIBILITY: If enriched targets are to be further analyzed by MS, please consider using alternative detergent free buffers after the initial wash steps to remove residual detergents that may interfere with MS analysis. This may include the initial buffer without detergent followed by 2-3 washes with a volatile salt-free buffer such as Ammonium Bicarbonate, Ammonium Formate, Triethylammonium Bicarbonate.

2.1. MagReSyn® Streptavidin Equilibration

MagReSyn® Streptavidin is supplied as a 10 mg.ml¹ suspension (80 mM Phosphate, pH 7.5, 150 mM NaCl, 1.5 mM EDTA, 0.05% Tween® 20, 0.02% sodium azide (NaN₃)). The shipping solution needs to be removed and the microparticles equilibrated in binding buffer (e.g. 80 mM sodium phosphate, pH 7.4–8.0, 150 mM NaCl, 0.05% Tween® 20) before use. Equilibrate aliquots of MagReSyn® Streptavidin for your requirements as outlined below. A minimum volume of 10 µl microparticle suspension is required per reaction to ensure a suitable pellet size for the aspiration of buffers.

- Resuspend MagReSyn® Streptavidin thoroughly by vortex mixing or inversion to ensure a homogenous suspension.
- 2) Transfer at least 10 μ l MagReSyn® Streptavidin to a new tube.
- 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 200 μl binding buffer.
- Place the tube on the magnetic separator and allow the microparticles to clear.
- Remove the binding buffer by aspiration with a pipette and repeat steps 5 and 6 twice for a total of three washes.

1

8) After removal of the binding buffer from step 5, MagReSyn® Streptavidin is ready for binding of your biotinylated molecules.

2.2. Immobilization of Biotinylated Oligonucleotide

- Calculate the amount of MagReSyn® Streptavidin microparticles required for your application and transfer to a clean tube.
- For example, 10 μl MagReSyn® Streptavidin microparticles (100 μg) is sufficient to bind ≥300 pmol biotinylated oligonucleotide (24 mer).
- 3) Add the biotinylated oligonucleotide to the equilibrated MagReSyn® Streptavidin from ${\bf 2.1.}$ Adjust the total reaction volume to at least 100 μ l with binding buffer and mix thoroughly by continuously agitating the tube.
- Allow the biotinylated oligonucleotide to bind to the microparticles for 15–30 min at room temperature.
- Place the tube on the magnetic separator and allow the microparticles to clear.
- 6) Aspirate the coupling supernatant with a pipette. The supernatant can either be discarded or used to quantify by difference the concentration of biotinylated oligonucleotide attached to the microparticles.
- 7) Remove any unbound oligonucleotide from the microparticles by washing the microparticles with $3 \times 200 \ \mu l$ binding buffer each.
- Following each wash, place the tube on the magnetic separator and allow the microparticles to clear.
- 9) Remove the supernatant with a pipette.
- 10) The supernatants from the wash steps can either be discarded or pooled with the coupling supernatant for the purpose of quantification.

2.3. Immobilization of Biotinylated Protein

- Calculate the amount of MagReSyn® Streptavidin microparticles required to immobilize your protein of interest. For example, 10 μl MagReSyn® Streptavidin microparticles (100 μg) is sufficient to bind approximately ≥30 μg Biotinylated IgG.
- 2) Add the biotinylated protein to the equilibrated MagReSyn® Streptavidin from 2.1. Adjust the total reaction volume to at least 100 μl with binding buffer and mix thoroughly by continuously agitating the tube.
- Allow the biotinylated protein to bind to the microparticles for 15–30 min at room temperature.
- Place the tube on the magnetic separator and allow the microparticles to clear.
- Remove the coupling supernatant with a pipette. The supernatant can either be discarded or used to quantify the concentration of biotinylated protein attached to the microparticles by difference.
- 6) Remove any unbound protein from the microparticles by washing with 3 x 200 μl binding buffer.
- Following each wash, place the tube on the magnetic separator and allow the microparticles to clear.
- 8) Aspirate the supernatant with a pipette.
- The supernatants from the wash steps can either be discarded or pooled together with the coupling supernatant for quantification.

3. Recommended Storage

MagReSyn® Streptavidin 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 (NaN₃) 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

MagReSyn® Streptavidin is compatible with samples containing the following buffering components:

Reagent	Concentration
Tween® 20	≤1%
Tris, Saline Sodium Citrate (SSC), Sodium phosphate	≤100 mM
NaCl	≤2 M

Should you wish to use the beads to capture binding partners for mass spectrometry analysis (e.g. BioID) please refer to MS compatible conditions available in relevant literature.

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 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	Incorrect binding	Increase pH of binding buffer to
biomolecules do	pH	pH 7.5-8.0
not bind to the	Insufficient	Incubate biotinylated molecules
microparticles as	reaction time	with the microparticles for at
expected		least 15–30 min.
	Interfering	Desalt or dialyze sample into
	compounds in	recommended binding buffer to
	sample prevent	remove media components or
	binding	other contaminants
	Insufficient	Increase amount of MagReSyn®
	microparticle	Streptavidin microparticles
	quantity	
	Biomolecule	Increase protein or
	content too low	oligonucleotide content by
		sample concentration or by
		preparing more starting material
	Inefficient	Refer to the troubleshooting
	biotinylation of	guide of the supplier of your
	target molecule	biotin-labelling kit or revisit the
		literature.
Non-specific	Non-specificity due	Increase NaCl concentration in
binding of non-	to ionic or	binding/wash buffers. Increase
biotinylated	electrostatic forces	the concentration of Tween® 20
molecules to the		in binding/wash buffers. Increase
microparticles		quantity of biotinylated
		molecule-containing sample.
	Insufficient	Increase number or volume of
	washing	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.