

MagReSyn[®] Protein A

Immobilized Protein A magnetic microparticles

Ordering Information		
Cat. No.	Quantity	
MR-PRA002	2 ml	
MR-PRA005	5 ml	
MR-PRA010	2 x 5 ml	

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® Protein A is a proprietary magnetic polymeric microparticle support that provides a simple and convenient method of capturing antibodies, suitable for the subsequent enrichment of various target molecules, as well as highly specific enrichment of various antibodies/immunoglobulins from biological samples. The ReSyn microparticle technology is differentiated from conventional solid or cracked bead technologies in that it is a hyperporous polymer network that allows penetration and binding of biomolecules throughout the volume of the microparticle. The exceptional Protein A capacity of the MagResyn® microparticles in turn translates to exceptionally high binding of target biomolecules. Recombinant Protein A (~41 kDa) is covalently linked to the magnetic microparticles, which allows for increased stability of the protein, potentially enabling application of the protein under non-standard conditions. The covalent linkage also helps to reduce the leaching of Protein A from the polymer support. MagReSyn® Protein A has superior antibody-binding capacity compared to alternative commercially available magnetic Protein A microparticles. Applications of Protein A microparticles include immunoglobulin depletion from serum samples, the isolation of antibodies and subsequent antibody target immunoprecipitation for downstream applications, e.g. analysis by mass spectrometry, immunoassay or electrophoresis.

1.2. Advantages of MagReSyn® Technology

The exceptional biological binding capacity of MagReSyn® allows for miniaturization of experimental protocols, as reduced volumes of the highly active functional microparticles can be used. This minimizes the volume of reagents required, facilitating the 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[®] 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 antibody specificity	High purity of target antibodies (≥ 97%) Minimizes/reduces requirement for additional purification steps
High antibody binding capacity >2.4 mg.ml ⁻¹ rabbit IgG	Miniaturization of experiments, reduced reagent volumes, high density of immobilized ligands
Rapid magnetic separation	Reduced particle carry-over Improved experimental reproducibility Rapid protocols
Multipoint covalent attachment of Protein A	Improved Protein A stability Reduced Protein A leaching Possibility of working in non-standard or denaturing conditions
Resistance 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 IgG molecules, Immunoprecipitation	
Matrix	Proprietary polymer	
Core	Iron (II, III) oxide (Magnetite)	
Functional group	Recombinant Protein A (~41 kDa)	
Binding capacity	≥2.4 mg.ml ⁻¹ IgG (Rabbit)	
Particle Size	~5–10 μM	
Formulation	1.5%: 15 mg.ml ⁻¹ in TBS [50 mM Tris pH 7.5, 150 mM NaCl, 0.025%	
	Tween [®] 20, 0.05% sodium azide (NaN ₃)]	
Stability	рН 2–10; 4–60°С	
Storage	Store at 4–8°C until expiry date on label	
	DO NOT EPEEZE	

1.4. Additional Equipment and Materials

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

2. Immunoglobulin Purification

Factors that may affect the attachment of antibodies include the isotype of the immunoglobulin, buffer composition and pH, and the presence contaminants/interfering compounds. The quantity of microparticles needs to be optimized for each individual application. We recommend the application of excess ligand to ensure saturation of the Protein A microparticles. The binding efficiency can be determined by comparing the ligand concentration before and after coupling. MagReSyn® Protein A is compatible with various commonly used buffers, including Tris and Phosphate. Recommended buffers include: Binding/wash buffer - TBS (50 mM Tris pH 7.5, 150 mM NaCl, 0.025% Tween® 20) or PBS (50 mM Phosphate pH 7.5, 150 mM NaCl, 0.025% Tween® 20); Elution Buffer (Native): 0.1 M glycine pH 2.5 or 2.5% acetic acid; Elution Buffer (Denaturing): SDS-PAGE electrophoresis buffer.

NOTE: All reagents should be freshly prepared and of analytical arade to ensure optimal performance. The procedures, methods and buffer solutions described below serve as an example and are not intended to be limiting. MagReSyn® Protein A is compatible with a range of different buffers for binding of antibodies. Achievable purity and yield are ligand dependent and experimental conditions should be optimized to ensure desired results.

2.1. MagReSyn® Protein A Equilibration

MagReSyn® Protein A is supplied as a 15 mg ml⁻¹ suspension in TBS (50 mM Tris pH 7.5, 150 mM NaCl with 0.025% Tween® 20, and 0.05% sodium azide as a preservative) and is suitable for purification of ≥2.4 mg.ml⁻¹ IgG (rabbit). The shipping solution needs to be removed and the microparticles equilibrated in binding buffer before use. Equilibrate aliquots of MagReSyn® Protein A for multiple binding reactions 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. The binding and elution protocols outlined below serve as examples and can be scaled to meet your requirements.

- 1) Resuspend MagReSyn® Protein A thoroughly by vortex mixing or inversion to ensure a homogenous suspension.
- Transfer 50 µl MagReSyn® Protein A (sufficient to capture ~120 µg of rabbit 2) IgG) to a new tube
- 3) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the shipping solution by aspiration with a pipette and discard in accordance with your local waste disposal legislation.
- 4) Wash/equilibrate the microparticles in 300 µl binding buffer (e.g. TBS or PBS), allow a minimum of 1 min for microparticle equilibration.
- 5) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the binding buffer by aspiration with a pipette and discard.
- 6) Repeat steps 4 and 5 twice (total of 3 washes).
- 7) After removal of the binding buffer from step 6, MagReSyn® Protein A is ready for binding of the target immunoglobulin.

2.2. Immunoglobulin (Ig) Purification from Serum or Culture Medium

- Calculate the volume of MagReSyn® Protein A microparticles required for the application and transfer to a clean tube. For example, 50 µl MagReSyn® Protein A microparticles (0.75 mg) is sufficient to bind \ge 120 µg Rabbit IgG.
- Dilute 10 μ l sample with a minimum of 90 μ l binding/wash buffer and mix by 2) vortexing for 3 s. Add the sample to the equilibrated MagReSvn® Protein A from 2.1. The sample volume may be adjusted to meet your requirements, but a minimum of nine parts binding buffer should be used to dilute the sample (e.g. 100 μl sample and 900 μl buffer may be used with 50 μl beads).
- Mix the sample with the microparticles by inversion or vortex mixing. Incubate at room temperature for a minimum of 10 min to ensure efficient binding 3) between protein A and the immunoglobulin. Binding efficiency is time and ligand dependent and incubation can be extended to 1 h to improve antibody vield.
- Place the tube on the magnetic separator and allow the microparticles to clear. 4) Aspirate the coupling supernatant with a pipette. The supernatant can either 5)
- be discarded or analyzed to determine Ig capacity. 6)
- Remove any unbound Ig and/or unwanted sample proteins from the microparticles by washing the microparticles with a minimum of 3 x 500 μl binding/wash buffer
- 7) Following each wash, place the tube on the magnetic separator and allow the microparticles to clear, aspirate the supernatant by pipette.
- 8) The supernatants from the wash steps can either be pooled with the coupling supernatant for quantification or gel electrophoresis if required.
- 9) The captured Ig can now be eluted from the microparticles as described in 2.3 or used for immunoprecipitation (3.1; from step 7)
- Optional: The bound antigen or antibody may be desalted or buffer exchanged at this point by washing in buffer/water suitable for downstream application. 10)

2.3. Recovery/Elution of Captured Immunoglobulins

Captured immunoglobulins can be eluted from the microparticles by the addition of low pH elution buffer, e.g. 0.1 M glycine pH 2.5 or 2.5% acetic acid.

- 1) Add 50 µl elution buffer to the MagReSyn® Protein A microparticles coated with captured Ig and mix thoroughly by continuous agitation of the tube. The elution volume can be adjusted up or down to suit user requirements; however, lower elution volumes might adversely affect the immunoglobulin yield.
- 2) Allow the captured Ig to elute from the microparticles for 1-2 min at room temperature.
- 3) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the solution containing the eluted Ig by aspiration with a pipette. This solution can be used to quantify the concentration of eluted lg, or analyzed by gel electrophoresis.
- 4) Steps 1-3 may be repeated to increase recovery of the eluted immunoglobulins.
- 5) Pool the eluates and neutralize the pH of the eluate by the addition of 5–7 μl of 5 M NaOH or 1 M Tris pH 9.0.
- The purified Ig can now be used for quantification or downstream analysis (e.g. 6) gel electrophoresis).

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3. Immunoprecipitation

MagReSyn® Protein A microparticles may further be used for antibody capture for direct or indirect immunoprecipitation (IP) reactions. For direct IP, a user-specified antibody (Ab) is first linked to the MagReSyn® Protein A microparticles through Protein A-antibody mediated binding. These immunoaffinity microparticles are subsequently used to specifically capture the antigen of interest (e.g. biomolecule, protein, protein complex) from a crude sample such as serum or cell lysate. An alternative method for IP is to first incubate the user-specified antibody with the sample containing the antigen to create an antigen-antibody complex. This is subsequently captured by application to the MagReSyn® Protein A microparticles. Indirect IP may further involve capturing the antigen-antibody immobilized by affinity capture to the MagReSyn® Protein A).

3.1. Immunoprecipitation (IP) Protocol

Due to the various permutations of IP, the protocol outlined below serves as an example only, and should be considered a guideline. Specific IP parameters that may vary, and which may therefore require optimization, include: quantity of antibodies and ligands, sample concentration, incubation time, temperature, and buffer composition. The protocol below can be adapted for direct and indirect IP and/or elution as required.

- Prepare and equilibrate MagReSyn[®] Protein A in accordance with 2.1., steps 1–6.
 After removal of the final binding/wash buffer, resuspend the MagReSyn[®]
- Protein A microparticles in 100 µl binding/wash buffer.
- 3) Add the capture antibody to the microparticle suspension (up to 120 µg per 50
- μl).
 4) Incubate at room temperature for 10–30 min to immobilize the capture antibody onto the microparticles.
- Place the tube on the magnetic separator and allow the microparticles to clear. Remove the antibody solution by aspiration with a pipette (discard or retain for quantification).
- 6) Wash the microparticles three times in 300 μl binding buffer (e.g. TBS or PBS).
- After the last wash, remove the tube from the magnet and add the sample containing the target antigen (minimum final volume of 500 µl for end-over-end mixing).
- 8) Mix well by gentle inversion and incubate at 2–8°C with continuous mixing. Optimal time for antibody-antigen interaction may vary (refer to your antibody manufacturer for the recommended incubation period; the usual range is from 1 h to overnight).
- Place the tube on the magnetic separator and allow the microparticles to clear. Remove the sample solution by aspiration with a pipette and discard or retain for downstream analysis.
- Wash the microparticles three times in 300 μl binding/wash buffer. The washes can be combined with the sample solution from step 9 for analysis.
- 11) Optional: The bound antigen or antibody may be desalted or buffer exchanged at this point by washing in buffer/water suitable for your downstream application such as mass spectrometry analysis, please contact info@resynbio.com for compatibility with down-stream analysis and clean-up methods.
- 12) The antibodies, antigens, or antibody-antigen complex may be eluted from the microparticles in accordance with **2.3**.

4. Recommended Storage

MagReSyn[®] Protein A is supplied as a suspension of 15 mg.ml⁻¹ in TBS (50 mM Tris pH 7.5, 150 mM NaCl), 0.025% Tween[®] 20, 0.05% 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.

5. Antibody Binding Guide

Species	Class of Antibody	Protein A binding
Human	Total IgG	Strong
	IgG _{1; 2; 4}	Strong
	IgG ₃ , IgM, IgA, Fab, ScFv	Weak
	IgD	No Binding
Mouse	Total IgG	Strong
	IgG _{2a; 2b; 3}	Strong
	IgG ₁	Weak
	IgM	No Binding
	Total IgG	Weak
Rat	IgG ₁	Weak
	IgG _{2a; 2b}	No Binding
	IgG _{2c}	Strong
Horse	Total IgG	Weak
	IgG(ab), IgG(c)	Weak
	IgG(T)	No Binding
Cow, Goat, Sheep	Total IgG	Weak
	IgG ₁	Weak
	IgG ₂	Strong
Rabbit, Guinea pig, Dog, Cat, Pig	Total IgG	Strong
Chicken	Total IgY	No Binding

6. Reagent Compatibility

MagReSyn® Protein A is compatible with several commonly used buffer components including:

Reagent	Concentration
Tween® 20	≤1%
Tris, Sodium phosphate, Triethanolamine	≤100 mM
NaCl	≤1 M

7. 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.

8. Troubleshooting Guide

Identified Problem	Possible Cause	Suggested Remedy
Antibodies do not	Incorrect binding pH	Ensure that the pH of the binding
bind to the		buffer is pH 7.4–8.0
microparticles as	Insufficient reaction	Increase incubation time of
expected	time	antibodies with the microparticles to
		1 h
	Interfering	Desalt or dialyze sample into
	compounds in sample	recommended binding buffer to
	prevent binding	remove media components or other
		contaminants
	Insufficient	Increase quantity of MagReSyn®
	microparticle	Protein A microparticles
	quantity	
	Biomolecule content	Increase antibody content by sample
	too low	concentration or prepare more
		starting material
	Incompatible	Protein A is specific for various, but
	antibody	not all, types of antibodies; Refer
		compatibility table, if not on table
		consult relevant scientific literature.
Non-specific binding	Non-specificity due	Increase NaCl concentration in
of biomolecules to	to ionic or	binding/wash and elution buffers.
the microparticles	electrostatic forces	Increase the concentration of Tween®
		20 in binding/wash buffers. Increase
		antibody to microparticle ratio.
	Insufficient washing	Increase number and/or volume of
		wash steps. Increase equilibration
		time. Carefully remove all remaining
		wash buffer from the microparticles
		to avoid possibility of carry-over.
Low recovery of	Elution conditions	Increase incubation time with elution
eluted antibody	too mild	buffer or use alternative elution
		buffer. Ensure elution buffer pH is
		below 3.0
Recovered	Elution conditions	Use alternative elution buffer or
protein/antibody	result in denaturation	neutralize eluted protein immediately
inactive after elution		by addition of 5 M NaOH or 1 M Tris
		рН 8.5–9

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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