MagReSyn® Protein G

Immobilized Protein G magnetic microparticles

Ordering Information	
Cat. No.	**Quantity**
MR-PRG002	2 ml
MR-PRG005	5 ml
MR-PRG010	2 x 5 ml

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® Protein G 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 hyper-porous polymer network that allows penetration and binding of biomolecules throughout the volume of the microparticle. The exceptional Protein G capacity of the MagReSyn® microparticles in turn translates to exceptionally high binding of target biomolecules. Recombinant Protein G (~32 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 G from the polymer support. MagReSyn® Protein G has superior antibody-binding capacity compared to alternative commercially available magnetic Protein G microparticles. Applications of Protein G 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 G	Improved Protein G stability Reduced Protein G 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 G (~32 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	pH 2–10; 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, 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 of 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 G microparticles. The binding efficiency can be determined by comparing the ligand concentration before and after coupling. MagReSyn® Protein G 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 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® Protein G 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.

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® Protein G Equilibration

MagReSyn® Protein G 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 G 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 G thoroughly by vortex mixing or inversion to ensure a homogenous suspension.
2) Transfer 50 µl MagReSyn® Protein G (sufficient to capture ~120 µg of rabbit 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 G is ready for binding of the target immunoglobulin.

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

1) Calculate the volume of MagReSyn® Protein G microparticles required for the application and transfer to a clean tube. For example, 50 µl MagReSyn® Protein G microparticles (0.75 mg) is sufficient to bind ≥ 120 µg Rabbit IgG.
2) Dilute 10 µl sample with a minimum of 90 µl binding/wash buffer and mix by vortexing for 3 s. Add the sample to the equilibrated MagReSyn® Protein G 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).
3) 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 between Protein G and the immunoglobulin. Binding efficiency is time and ligand dependent and incubation can be extended to 1 h to improve antibody yield.
4) Place the tube on the magnetic separator and allow the microparticles to clear.
5) Aspirate the coupling supernatant with a pipette. The supernatant can either 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**)
10) *Optional*: The bound antigen or antibody may be desalted or buffer exchanged at this point by washing in buffer/water suitable for downstream application.

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 G 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 Ig, or analyzed by gel electrophoresis..
4) Steps 1–3 may be repeated to increase recovery of the eluted immunoglobulins.

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

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.
6) The purified Ig can now be used for quantification or downstream analysis (e.g. gel electrophoresis).

3. Immunoprecipitation

MagReSyn® Protein G 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 G microparticles through Protein G-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 G microparticles. Indirect IP may further involve capturing the antigen-antibody complex from a sample through use of a secondary antibody (an anti-antibody immobilized by affinity capture to the MagReSyn® Protein G).

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.

1) Prepare and equilibrate MagReSyn® Protein G in accordance with **2.1**., steps 1–6.
2) After removal of the final binding/wash buffer, resuspend the MagReSyn® Protein G 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.
5) 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).
7) 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).
9) 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.
10) 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 G 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 (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.

5. Antibody Binding Guide

Species	Class of Antibody	Protein G binding
Human	Total IgG	Strong
	$IgG_{1, 2, 3, 4}$	Strong
	Fab; F(ab')2; Fc	Weak
	IgA; IgD; IgE; IgM; ScFv	No Binding
Mouse	Total IgG	Strong
	$IgG_{2a, 2b, 3}$	Strong
	IgG_1	Medium
	IgM	No Binding
Rat	Total IgG	Medium
	IgG_1	Medium
	IgG_{2b}	Weak
	$IgG_{2a, 2c}$	Strong
Horse	Total IgG	Strong
	IgG(ab), IgG(c)	No Binding
	IgG(T)	Strong
Cow, Goat, Sheep	Total IgG	Strong
	IgG_1	Strong
	IgG_2	Strong
Guinea pig, Dog, Cat, Pig	Total IgG	Weak
Rabbit	Total IgG	Strong
Chicken	Total IgY	No Binding

6. Reagent Compatibility

MagReSyn® Protein G 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 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 bind to the microparticles as expected	Incorrect binding pH	Ensure that the pH of the binding buffer is pH 7.4–8.0
	Insufficient reaction time	Increase incubation time of antibodies with the microparticles to 1 h
	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 quantity of MagReSyn® Protein G microparticles
	Biomolecule content too low	Increase antibody content by sample concentration or prepare more starting material
	Incompatible antibody	Protein G is specific for various, but not all, types of antibodies; Refer compatibility table, if not on table consult relevant scientific literature.
Non-specific binding of biomolecules to the microparticles	Non-specificity due to ionic or electrostatic forces	Increase NaCl concentration in binding/wash and elution buffers. 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 eluted antibody	Elution conditions too mild	Increase incubation time with elution buffer or use alternative elution buffer. Ensure elution buffer pH is below 3.0
Recovered protein/antibody inactive after elution	Elution conditions result in denaturation	Use alternative elution buffer or neutralize eluted protein immediately by addition of 5 M NaOH or 1 M Tris pH 8.5–9

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