MagReSyn® SAX

Strong anion exchange magnetic microparticles

Ordering Information	
Cat. No.	**Quantity**
MR-SAX002	2 ml
MR-SAX005	5 ml
MR-SAX010	2 x 5 ml

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® SAX (strong anion exchanger) is a proprietary magnetic polymeric microparticle support designed for the isolation, purification and recovery of biomolecules such as proteins/peptides, enzymes, antibodies, DNA or RNA by exploiting differences in net charge. These biomolecules usually exhibit a net negative charge and are readily and reversibly adsorbed to the positively charged MagReSyn® SAX microparticles. The ReSyn technology is differentiated from conventional solid or cracked bead 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 exceptional capacity for the binding of biomolecules. A high functional group density allows multi-point attachment and binding, resulting in strong ionic binding of target biomolecules. This advancement in capacity translates into orders of magnitude improvement in performance over alternative technologies. MagReSyn® SAX is ideal for the fractionation of complex biological mixtures (e.g. culture supernatants, serum or plasma) prior to 1D or 2D electrophoresis, HPLC or mass spectrometry.

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 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 target proteins of exceptional purity to meet your stringent R&D requirements.

MagReSyn® Technology Advantages	End-user Benefits
High biological binding capacity	Miniaturization of experiments Reduced reagent volumes Increased concentration of eluted biological Improved recovery of target biologicals
Rapid magnetic separation	Reduced particle carry-over Improved experimental reproducibility Rapid protocols
Resistant to oxidation (rust)	Reduced sample contamination Improved shelf life

1.3. Product Information

Product Specifications	
Description	Iron oxide-containing magnetic polymer microparticles
Application	Fractionation/purification of proteins, peptides, enzymes, antibodies, DNA, RNA
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Functional group	Quaternary ammonium
Binding capacity	≥20 $mg.ml^{-1}$ (BSA)
Particle Size	~5–20 µM
Formulation	2%: 20 $mg.ml^{-1}$ suspension in 20% ethanol
Stability	pH 3.5–10; 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 procedure

For efficient biomolecule adsorption in anion exchange chromatography, the ionic strength of the binding buffer needs to be low and the pH of the binding solution should be at least one unit above the isoelectric point (pI) of the biomolecule of interest. At a pH above the pI, the biomolecule will be negatively charged and will adsorb to the positive groups (quaternary ammonium) on the anion exchanger (Fig. 1).

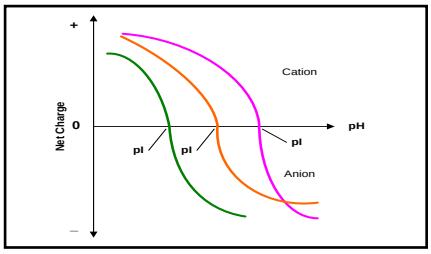

Fig. 1: Theoretical titration curves of three different biomolecules indicating the pH-dependence of the net surface charge.

NOTE: All reagents should be freshly prepared and of analytical grade to ensure optimal performance. The buffer solutions described below serve as an example and are not intended to be limiting. MagReSyn® SAX is compatible with a range of different buffers for binding/adsorption and elution/desorption.

2.1. Equilibration of MagReSyn® SAX

MagReSyn® SAX is supplied as a 20 $mg.ml^{-1}$ suspension in 20% ethanol. The shipping solution needs to be removed and the microparticles equilibrated in binding buffer (e.g. 50 mM Tris, pH 8.0) before use. Equilibrate sufficient aliquots of MagReSyn® SAX for multiple binding reactions as outlined below. This protocol can be scaled up or down to suit your requirements - the current protocol is estimated for the binding of ~1 mg of protein.

1) Resuspend MagReSyn® SAX thoroughly by vortex mixing for 3 s to ensure a homogenous suspension.
2) Transfer 50 µl (1 mg) MagReSyn® 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.
4) Wash/equilibrate the microparticles for 1 min in 250 µl binding buffer, e.g. 50 mM Tris or triethanolamine, pH 8.0.
5) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the binding buffer by aspiration with a pipette.
6) Repeat steps 4 and 5 twice for a total of three washes.
7) After removal of the binding buffer, MagReSyn® SAX is ready for the binding of biological molecules.

2.2. Protein Binding Procedure

After protein sample preparation, it is recommended that all samples be filtered through a 0.2 µm filter or centrifuged at 10,000 x g for 5 min to remove particulates that could potentially interfere with biomolecule binding.

1) Add the protein sample containing up to 1 mg total protein (in a suitable binding buffer) to the equilibrated MagReSyn® SAX. Adjust the total reaction volume to a minimum of 250 µl with binding buffer and mix thoroughly by pipetting or vortexing for 3 s. *Note: to ensure binding it is important that your protein be in a compatible binding buffer; your protein sample may be buffer-exchanged into a suitable buffer using e.g. a PD-10 column or similar.*
2) Allow proteins to bind to the microparticles for 5–10 min at room temperature. Mix continuously to ensure good sample-microparticle interaction during the binding procedure.
3) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the supernatant by pipette aspiration. The supernatant can either be discarded or used for protein quantification.
4) Add a minimum of 250 µl binding buffer to wash the microparticles and resuspend by vortex mixing for 3 s.
5) Recover the microparticles on the magnetic separator. Invert the magnet with the tube in place to collect any microparticles that may be caught in the tube lid.
6) Remove the supernatant with a pipette. The supernatant can either be discarded or used for protein quantification.
7) Repeat steps 4–6 twice for a total of three washes.

2.3. Protein Elution Procedure

The adsorbed protein/peptide mixture can be fractionated by stepwise elution with buffer solutions of either an increasing salt concentration or decreasing pH.

2.3.1. Fractionation: Increasing Salt Concentration

1) Resuspend the microparticles with the adsorbed protein/peptide mixture from **2.2** in 50 µl elution buffer (e.g. 50 mM Tris pH 8.0, 50 mM NaCl) and allow desorption at room temperature for 2 min.
2) Recover the microparticles on the magnetic separator, aspirate the supernatant with a pipette. Keep the supernatant for protein quantification or further analysis.
3) Repeat steps 1 and 2 twice to improve recovery of the fractionated proteins. Pool eluates of equivalent salt concentration for further analysis.
4) Repeat steps 1–3 using an elution buffer consisting of increasing salt concentrations (e.g. 50 mM Tris pH 8.0, 100, 150, 200, or 250 mM NaCl, etc.). *Note: the salt concentration of the elution buffer may be increased up to 2 M NaCl if required.*
5) The eluted samples contain differentially fractionated proteins/peptides.

2.3.2. Fractionation: Decreasing pH

1) Resuspend the microparticles with the adsorbed protein/peptide mixture from **2.2** in 50 µl of elution buffer (e.g. 50 mM Tris, pH 7.5) and allow desorption at room temperature for 2 min.
2) Recover the microparticles on the magnetic separator, aspirate the supernatant with a pipette. Keep the supernatant for protein quantification or further analysis.
3) Repeat steps 1 and 2 twice to improve recovery of the fractionated proteins. Pool eluates of equivalent pH for further analysis.
4) Repeat steps 1–3 using an elution buffer of decreasing pH (e.g. 50 mM Tris at pH 7.0, pH 6.5, etc.). The eluted samples now contain differential fractions of the original protein/peptide mixture.

3. Recommended Storage

MagReSyn® SAX is supplied as a suspension of 20 mg.ml^{-1} 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 and 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.

5. Troubleshooting Guide

Identified Problem	Possible Cause	Suggested Remedy
Proteins do not bind to the microparticles as expected	Weak ionic interaction	Decrease ionic strength (salt concentration) of binding buffer
	Incorrect binding pH	Check and adjust pH of binding buffer Check electrode calibration
	Protein of interest degraded	Add protease inhibitors to protein extract
	Interfering compounds in sample prevent binding	Desalt or dialyze sample into recommended binding buffer to remove media components/other contaminants
	Insufficient particle quantity	Increase amount of MagReSyn® SAX particles
	Protein content too low	Increase protein content by sample concentration or prepare more starting material
Low recovery of proteins during elution	Ionic interaction too strong	Increase NaCl concentration in elution buffer
	pH too alkaline	Decrease pH of elution buffer to reduce strength of ionic interaction between microparticle and biomolecules
	Protein degradation occurs during purification	Add protease inhibitors to samples and buffers to prevent proteolysis Use freshly prepared samples and solutions Reduce sample preparation times Decrease working temperature where possible
	Protein may be unstable or inactive in elution buffer	Determine optimal pH and salt stability of protein of interest
Insufficient protein fractionation	Protein carry-over between fractions	Use lower incremental increases in NaCl concentrations between fractions. Include wash steps between each NaCl elution
	Protein of interest has similar net charge to contaminating proteins	Consider optimising sample preparation steps Optimise NaCl concentration and pH of elution buffer
Target protein inactive after elution	Interference by buffering agents or salts	Remove buffer salts by dialysis, filtration, precipitation or size-exclusion chromatography post fractionation. Decrease buffer concentration for elution or elute in acidic buffer, e.g. citrate pH 3–4; Immediately increase pH after elution using NaOH or suitable buffer
Target protein is incompatible with downstream applications	Interference by NaCl or buffering agents used in elution	Remove agents by dialysis, filtration, precipitation or size-exclusion chromatography

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

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