MagReSyn® HILIC

Magnetic particles for hydrophilic interaction chromatography

Ordering Information	
Cat. No.	**Quantity**
MR-HLC002	2 ml
MR-HLC005	5 ml
MR-HLC010	2 x 5 ml

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® HILIC (hydrophilic interaction chromatography) is a proprietary magnetic polymeric microparticle support designed for the solid phase extraction (SPE) and recovery of biomolecules from common sample contaminants that may interfere with downstream analytical procedures, primarily aimed at sample preparation for Mass-Spectrometry. The ultra-capacity MagReSyn® HILIC polymer microparticles contain mixed-mode functionality leading to high affinity for the biomolecules, with subsequent elution in low volumes, enabling concentration during sample processing. MagReSyn® HILIC is ideal for sample preparation of complex biological mixtures (e.g. culture supernatants, tissues extracts, serum or plasma), from routine contaminants such as SDS, CHAPS, NP-40 and Urea prior to analysis of samples by electrophoresis, HPLC and mass spectrometry. Magnetization further allows the automation of routine Mass-Spectrometry clean-up workflows, improving reproducibility and data quality.

1.2. Advantages of MagReSyn® Technology

The advanced ReSyn polymer technology allows for the engineering of highly specific microparticles to address the limitations of current microparticle-based technologies. 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 are engineered with multiple chemical residues to deliver target protein of exceptional purity to meet your stringent R&D and mass spectrometry sample requirements.

MagReSyn® Technology Advantages	End-user Benefits
High biological binding capacity	Miniaturization of experiments Reduced reagent volumes Increased concentration of eluted biological (reduced volume elution)
High functional group density	High recovery of biomolecules Compatibility with common contaminants such as detergents, chaotropes and salts
Rapid magnetic separation	Reduced particle carry-over Improved experimental reproducibility Rapid protocols High-throughput compatible
Resistant to oxidation (rust)	Reduced sample contamination Longer shelf life

1.3. Product Information

Product Specifications	
Description	Iron oxide-containing magnetic polymer microparticles
Application	Protein purification, proteomics, mass spectrometry sample preparation
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Function Group	HILIC, Mixed Mode
Particle Size	~5–10 µM
Formulation	2%: 20 mg.ml^{-1} suspension in 20% EtOH
Stability	pH 2-12; 4 to 60ºC
Storage	Store at 4–8ºC until expiry date on label **DO NOT FREEZE**

1.4. Additional Equipment and Materials

Magnetic separator, vortex mixer, end-over-end mixer, buffers and solutions (refer 4).

2. Binding and Elution Procedures

HILIC (hydrophilic interaction liquid chromatography) is the process whereby amphiphilic biomolecules become trapped between a water-rich layer formed on the surface of a stationary phase by applying an organic mobile phase. This mechanism is used to separate biomolecules from less polar compounds. Hydrogen and weak electrostatic interactions have further been implicated in retention of biomolecules at the aqueous layer, while ionic additives such as ammonium acetate and ammonium formate are typically used to control the pH and ionic strength of the sample.

NOTE: The current protocol is suitable for clean-up of reduced and alkylated proteins prior to tryptic digestion for MS analysis, and has not yet been evaluated for the clean-up of peptides. For an example of a suitable workflow for MS please refer to the application note on our website (ASMS 2017 poster).

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. Purity and yield are dependent on experimental conditions and these should be optimized for each application. Please carefully read instructions prior to use.

2.1. Equilibration of MagReSyn® HILIC

MagReSyn® HILIC is supplied as a 20 mg.ml^{-1} suspension in 20% ethanol. The shipping solution needs to be removed and the microparticles equilibrated before use. The recommended protocol may be scaled up or down to suit your requirements, but a minimum volume of 10 µl microparticle suspension is required per reaction to ensure a suitable magnetic microparticle pellet for the aspiration of buffers. **The current protocol is optimized for the binding of 50 µg of total protein to 500 µg of beads (1:10 protein to bead ratio), within a volume of 25 µl of sample (minimum for this protocol). The procedure has been shown to be effective down to concentrations as low as 20 µg of total protein.**

1) Resuspend MagReSyn® HILIC thoroughly by vortex mixing for 3 s to ensure a homogenous suspension.
2) Transfer 25 µl (500 µg) MagReSyn® to a new 2 ml microcentrifuge tube (recommend low protein binding to improve sample recovery).
3) Place the tube on the magnetic separator and allow 10 s for the microparticles to clear.
4) Remove the shipping solution by aspiration with a pipette and discard.
5) Wash and equilibrate the microparticles in 250 µl Equilibration Buffer (refer section **4**), with gentle agitation for 1 min.
6) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the wash buffer by aspiration with a pipette and discard.
7) Repeat steps 5 and 6.
8) After removal of the binding buffer, MagReSyn® HILIC is ready for the binding of proteins.

2.2. Protein Binding Procedure

Proteins should be extracted and solubilized in low molarity buffer (e.g. 50 mM Tris pH 8.0) containing a suitable detergent or chaotrope for increasing the solubility of your protein sample. The protocol has been shown to effectively remove up to **5% SDS**, **2% CHAPS**, **1% NP40**, or **8 M Urea** from cell lysates in 50 mM Tris pH 8.0 or PBS; making these compatible agents for protein solubilization (refer section **6**). **Where enzymatic digestion is required prior to Mass Spectrometry analysis, samples should be reduced and alkylated prior to clean-up on MagReSyn® HILIC.**

1) Adjust the sample to a minimum of 25 µl with protein solubilization buffer, and add equivalent volume (1:1) binding buffer (refer section **4**), to a final volume of 50 µl (minimum). ***Note: the binding procedure is suitable for samples of increased volumes (e.g. in the event you have a lower protein concentration).***
2) Add the sample to the equilibrated MagReSyn® HILIC and mix thoroughly by pipette mixing.
3) Allow proteins to bind to microparticles for 30 min. Mix gently and continuously to ensure good sample microparticle interaction during the binding procedure. Excessive mixing can result in microparticles drying on tube side walls and result in poor protein recovery.
4) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the supernatant by pipette aspiration (***NOTE: Supernatant may be analysed to determine the presence of unbound protein***).
5) Resuspend the beads in 200 µl Wash Buffer (Refer section **4**) and mix thoroughly for 1 minute.
6) Recover the microparticles on the magnetic separator. Remove the supernatant by pipette aspiration.
7) Repeat wash steps 5-6.
8) Proceed to **2.3** for Protein Digestion, or proceed to alternate procedure **2.4** for Protein Elution.

2.3 Protein Digestion Procedure

Proteins can be digested using enzymes such as trypsin by applying the MagReSyn® HILIC microparticles with bound proteins directly to a trypsin solution. Resulting peptides can be analyzed via mass spectrometry without the need for further purification. ***NOTE: to ensure efficient digestion, proteins should be reduced and alkylated (as per standard protocols) prior to HILIC purification***.

1) Resuspend the microparticles with the adsorbed protein mix from **2.2** in 50-200 µl suitable digestion buffer, e.g. 5-10 mM ammonium bicarbonate containing a digestion enzyme. In the case of sequencing grade trypsin we recommend an enzyme to protein ratio of 1:10.
2) Incubate sample at suitable temperature and time period as per enzyme specifications. In the case of sequencing grade trypsin we recommend incubation at 37°C for 4 hr. Mix continuously to ensure microparticles remain in suspension. Excessive mixing can result in microparticles drying on tube side walls and result in poor protein recovery.
3) Recover the microparticles on the magnetic separator, aspirate the supernatant containing peptides with a pipette.
4) Proceed with vacuum drying or lyophilization to concentrate your peptide sample as required.
5) Resuspend peptides as necessary for MS analysis, e.g. 2% acetonitrile with 0.2% formic acid.
6) Proceed to mass spectrometry analysis.

2.4. Protein Elution Procedure (Alternate to 2.3 above)

1) Resuspend the microparticles with the adsorbed protein mixture (from **2.2**) in 50 µl Elution solution (refer section **4**) and allow desorption for 5 min at room temperature. Mix continuously to ensure microparticles remain in suspension during elution.
2) Recover the microparticles on a magnetic separator and aspirate the supernatant (containing protein) with a pipette.
3) Steps 1-2 may be repeated to improve protein recovery.
4) Pool eluates and concentrate or dry as necessary using lyophilization or vacuum filtration.

NOTE: For samples with low protein content, concentration of the sample (e.g. vacuum drying or lyophilization) may be required prior to analysis.

3. Recommended Storage

MagReSyn® HILIC 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. Recommended Buffers

Equilibration Buffer: 100 mM ammonium acetate , pH 4.5, 15% acetonitrile
Binding Buffer: 200 mM ammonium acetate , pH 4.5, 30% acetonitrile
Wash Buffer: 95% acetonitrile (5% water)
Elution solution: 100% ethanol
Acid Solution: 10% formic acid

5. 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.

6. Compatibility

The current protocol has successfully been used to purify protein from mammalian cell lysate extracted in Phosphate Buffered Saline (PBS) or Tris 50mM pH 8.0 containing either of the following components:

- 8 M Urea
- 4% SDS
- 2% CHAPS
- 1% NP40

NOTE: The compatibility list is not comprehensive and may be adjusted from time to time. Please download the latest version of this protocol to review updated list of compatibilities.

7. Incompatibility

The current protocol is not suitable for purifying protein from mammalian cell lysate containing the following components:

- 6 M Guanidium HCl in 50 mM Tris, pH 8.0

NOTE: The list is not comprehensive and the conditions for binding may not yet have been determined. Please request a sample if you are unsure as to whether this product will work for your application.

8. Troubleshooting Guide

Identified Problem	Possible Cause	Suggested Remedy
Low protein binding	Low acetonitrile concentration	Ensure final acetonitrile concentration of samples prior binding is ≥15% , Evaluate increased acetonitrile concentrations of up to 80%. Monitor for potential protein precipitation at increased acetonitrile concentration.
	High salt content	Reduce salt concentration to increase adsorption. Salt/buffer concentration should not exceed 100 mM
	Incompatible agents in sample	Adjust sample preparation to include only compatible chaotropic agents and buffers for HILIC. Dilute chaotropic or solubilization agents prior to clean-up with MagReSyn® HILIC
	Insufficient mixing	Make use of 2 ml Protein LoBind tubes to ensure easy mixing and reduced adsorption of microparticles to tube side walls
Low sequence coverage or low mass spectrometry signal post digestion	Incomplete digestion	Ensure optimal preparation and conditions for digestion: ensure samples are reduced and alkylated efficiently; pH of digestion =8; suitable protein to enzyme ratio; digestion temperature is 37°C. If necessary increase digest time.
	Low peptide recovery or incomplete elution	Wash microparticles post-digestion to improve recovery. Ensure protein bound to microparticles by gel analysis of binding and elution samples.
	Low protein concentration	Reduce sample volumes prior mass spectrometric analysis by vacuum drying or lyophilization.
Incomplete removal of denaturants	Carry-over of impurities	More stringent washing procedure required using additional acetonitrile washes.
Incomplete elution of protein during alternate procedure (refer 2.4)	Tight binding due to use of high molarity buffer prevents elution of protein	Reduce molarity of binding buffer to between 20 and 50 mM to reduce bead-protein interaction.

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