MagReSyn® Ti-IMAC

Titanium ion (Ti^{4+}) functional magnetic microparticles

Ordering Information	
Cat. No.	**Quantity**
MR-TIM002	2 ml
MR-TIM005	5 ml
MR-TIM010	2 x 5 ml

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® Ti-IMAC (immobilized metal affinity chromatography using titanium ions) is a proprietary magnetic polymeric microparticle support that provides a simple, convenient, efficient and specific method of phosphopeptide enrichment from e.g. trypsin-digested protein mixtures. The product consists of titanium ions (Ti^{4+}) chelated to the ReSyn polymer microparticles. The superior features of the polymer technology have been harnessed to engineer a highly specific product for the enrichment of phosphopeptides suitable for mass spectrometry-based proteomics applications.

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 bead-based technologies. MagReSyn® microparticles are separated rapidly (<10 sec) 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, and suitability for automation. The microparticles and buffers are engineered to deliver target phosphopeptides of exceptional purity to meet your stringent R&D requirements.

MagReSyn® Technology Advantages	End-user Benefits
High phosphopeptide purity	High selectivity & increased sample coverage
Rapid magnetic separation	Improved research efficiency Reduced particle carry-over Improved experimental reproducibility Rapid protocols High-throughput compatible Compatible with automation
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 phosphopeptides
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Functional group	Ti^{4+}
Particle Size	~5–10 µM
Formulation	2%: 20 $mg.ml^{-1}$ suspension in 20% ethanol
Stability	pH 2.5–12; 4–60^{o}C
Storage	Store at 4–8^{o}C until expiry date on label **DO NOT FREEZE**

1.4. Additional Equipment and Materials Required

Magnetic separator, Vortex mixer, Buffers and solutions

2. Binding and Elution Procedure

Factors that may affect the binding of phosphopeptides include buffer composition and pH, and the presence of contaminants such as **DNA** or other interfering compounds. DNA degradation in your lysate (with e.g. Benzonase®) is recommended (sonication may be insufficient to degrade DNA). We recommend **de-salting** of your samples prior to enrichment to remove interfering compounds using e.g. Waters Sep-Pak C18 or Oasis HLB cartridges. The quantity of microparticles required may require optimization for your application, primarily peptide:bead ratio to ensure optimal recovery and specificity.

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® Ti-IMAC is compatible with a range of different buffers for phosphopeptide enrichment. Achievable purity and yield are dependent on experimental conditions and these should be optimized for each particular application.

2.1. Equilibration of MagReSyn® Ti-IMAC

MagReSyn® Ti-IMAC is supplied as a 20 $mg.ml^{-1}$ suspension in 20% ethanol. The shipping solution needs to be removed and the microparticles washed and equilibrated before use. A minimum starting volume of 20 µl microparticle suspension is required per reaction to ensure a suitable pellet size for the aspiration of buffers and automation of this protocol. **The current protocol is sufficient for the purification of phosphopeptides from ~500 µg of total protein digest**. The protocol may be adapted for input material using the following adjustments to the current protocol:

Protein Digest	Bead Quantity	Ratio Bead:Peptide	Equilibration Volume (µl)	Wash Volume (µl)
20 µg	20 µl: 0.4 mg	20	200	100
50 µg	20 µl: 0.4 mg	8	200	100
100 µg	20 µl: 0.4 mg	4	200	100
200 µg	40 µl: 0.8 mg	4	200	100
500 µg	100 µl: 2 mg	4	200	100
1 mg	200 µl: 4 mg	4	200	200
2 mg	400 µl: 8 mg	4	400	400

NOTE: Although this is the recommended protocol, enrichment may be sample dependant. As an example: you can decrease the bead to protein ratio for samples with low phosphopeptide abundance (i.e. use 1 mg of beads for 500 ug of protein, a protein:bead ratio of 1:2). The ideal ratio should be empirically determined for your sample.

1) Resuspend MagReSyn® Ti-IMAC thoroughly by vortex mixing or inversion to ensure a homogenous suspension.
2) Transfer 100 µl (2 mg of beads, a 1:4 protein to bead ratio) MagReSyn® Ti-IMAC to a 2 ml micro-centrifuge tube. *NOTE: 2 ml microcentrifuge tubes provide better mixing and agitation of microparticles during mixing than 1.5 ml tubes.*

Patent numbers WO2009057049; IN267234; US9574054; EP2205662 (DK, FR, GB, NL, FI, DK, AT, IT, CH, BE, IE, ES, FR, DE, SE); CN101874061; CA2703478; BR-PI0819130-1; JP五四四二六二四

3) Place the tube on a magnetic separator and allow 10 sec for the microparticles to clear.
4) Remove the shipping solution by aspiration with a pipette and discard.
5) Equilibrate the microparticles in 200 µl *Loading Buffer* (0,1M glycolic acid in 80% ACN, 5% TFA), allow 60 sec for equilibration. *NOTE: Adjusting the glycolic acid concentration can have an effect on capacity, specificity, and selectivity (e.g. singly vs multi phosphorylated peptides) of enrichment. For best results we recommend evaluating a range of glycolic acid concentrations prior to embarking on a large scale study as enrichment efficiency can be affected by sample source and purity.*
6) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the *loading buffer* by aspiration with a pipette.
7) Repeat steps 5 and 6 twice for a total of three equilibrations.
8) After removal of the *Loading Buffer*, MagReSyn® Ti-IMAC is ready for binding of the target phosphopeptides.

2.2. Phosphopeptide Enrichment Procedure

1) Resuspend 500 µg de-salted protein digest in 200 µl *loading buffer* (refer to **Section 4**) and mix by vortexing.
2) Centrifuge at 10000 xg for 5 min at 4°C to remove any insoluble material.
3) Transfer supernatant to the equilibrated MagReSyn® Ti-IMAC microparticle pellet from **2.1**.
4) Resuspend the microparticles in the peptide sample by vortexing or pipette aspiration.
5) Incubate for 20 min at room temperature with continuous mixing (e.g. slow vortexing) to ensure adequate sample and microparticle interaction.
6) Place the tube on the magnetic separator and allow the microparticles to clear. Remove and discard the coupling supernatant by aspiration with a pipette.
7) Remove unbound sample by washing with 100 µl of loading buffer for 2 min with gentle agitation.
8) Place the tube on a magnetic separator and allow 10 sec for the microparticles to clear. Remove the supernatant and discard.
9) Remove non-specifically bound peptides by resuspending the microparticles in 100 µl *Wash Buffer 1* (aqueous solution of 80% ACN and 1% TFA) for 2 min with gentle agitation.
10) Place the tube on a magnetic separator and allow 10 sec for the microparticles to clear. Remove the supernatant and discard.
11) Perform an additional 2 min wash using 100 µl of *Wash Buffer 2* (aqueous solution of 10% ACN and 0.2% TFA with magnetic recovery as above).
12) Elute the bound phosphopeptides from the microparticles by adding 150 µl elution buffer (1% NH_4OH) for 10 min. Ensure that the microparticles remain in suspension by constant gentle agitation during the elution step.
13) Place the tube on a magnetic separator and allow 5 to 10 sec to clear.
14) Transfer the supernatant (eluted phosphopeptides) to a 1.5 ml Protein LoBind tubes (Eppendorf®) containing 50 µl of 10% Formic Acid
15) Repeat elution steps 12 to 14 for improved recovery.
16) Pool eluates for a total volume of 400 µl.
17) Lyophilize or vacuum dry eluates from frozen (samples frozen at -80°C for 30 minutes).
18) Analyze the sample by mass spectrometry. Samples can be de-salted prior analysis using C18 SPE or in-line C18 trap cartridge used in a typical pre-concentrations LCMS setup.

3. Recommended Storage

MagReSyn® Ti-IMAC 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

Loading buffer: 0.1 M glycolic acid in 80% acetonitrile (ACN) and 5% trifluoroacetic acid (TFA)
Wash Buffer 1: 80% ACN, 1% TFA
Wash Buffer 2: 10% ACN, 0.2% TFA
Elution Buffer: 1% NH_4OH

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 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. Troubleshooting Guide

Identified Problem	Possible Cause	Suggested Remedy
Phosphopeptides do not bind to the microparticles as expected	Insufficient reaction time	Extend the sample-bead incubation time to 30 min
	Interfering compounds in sample prevent binding	Treat with DNAse (e.g. Benzonase®) and desalt sample into recommended binding buffer to remove potential interfering components such as nucleic acids, buffers and salts. Contaminants may further result in low specificity of enrichment.
	Insufficient bead quantity	Increase quantity of IMAC beads (increase bead:peptide ratio)
Non-specific binding of peptides	Insufficient competition for phosphopeptide binding	Increase molarity of glycolic acid in binding buffer **or** decrease bead to protein ratio to improve competition
	Insufficient wash volume	Increase volume of wash buffer (up to 500 µl) and ensure proper mixing
	Insufficient wash time	Increase time for each wash step to improve removal of non-specifically bound peptides
	Try alternate wash buffer	**Wash Buffer 1**: 60% ACN, 1% TFA, 200mM NaCl **Wash Buffer 2**: 60% ACN, 1% TFA
Low recovery/signal of phosphopeptides	Try alternate elution buffer	Confirm concentration of NH4OH is 1%. Increase concentration to 4% and add ACN to a final of 40%.
	Re-apply existing beads to sample	Retain supernatant from step 6 (section 2.2) and re-load on same of re-equilibrated Zr-IMAC beads (this approach has been shown to improve coverage with Ti-IMAC HP, not currently for Ti-IMAC)
	Sample concentration too dilute	Concentrate samples by lyophilization or vacuum centrifugation prior to MS analysis
	Lower than expected MS signal	Desalt phosphopeptides by HPLC (e.g. C18 RP-trap column or similar) or using solid-phase extraction (OLIGO®-R3 by Life Technologies or equivalent). Reduce polymer bead exposure time to elution buffers.
Low recovery from TMT labelling applications	Incompatibility with samples or reagents	Please refer to product citations for recommendations or contact us.

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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