MagReSyn® ZrO_2
Zirconium dioxide functional magnetic microparticles

Ordering Information	
Cat. No.	**Quantity**
MR-ZRD002	2 ml
MR-ZRD005	5 ml
MR-ZRD010	2 x 5 ml

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® ZrO_2 (zirconium dioxide) 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 zirconium dioxide nanoparticles attached to the ReSyn polymer microparticle support. The superior features of the polymer technology have been harnessed to engineer a highly specific product with no apparent bias towards single or multi-phosphorylated peptides. This has resulted in a product that is highly suitable for single-step enrichment of phosphorylated peptides 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 limitations in current microparticle-based technologies. The compressibility of the microparticles reduces the interstitial spaces between the beads during washing and elution, leading to the increased efficiency of these steps and concomitant higher purity of the target phosphopeptides. 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 buffers are engineered to deliver target phosphopeptides of exceptional purity to meet your stringent R&D requirements.

MagReSyn® Technology Advantages	End-user Benefits
Exceptional specificity towards phosphopeptides	High purity of target phosphopeptides reduces the requirement for additional or complementary purification procedures. Improved data quality
Reduced bias for mono- or multi-phosphorylated peptides	Reduced requirement for complementary purification procedures Improved research efficiency
Rapid magnetic separation	Reduced particle carry-over Improved experimental reproducibility Rapid protocols High-throughput compatible
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	Zirconium dioxide nanoparticles
Binding capacity	≥250 µg phosphopeptides per ml suspension
Particle Size	~5-10 µM
Formulation	5%: 50 $mg.ml^{-1}$ suspension in 20% ethanol
Stability	pH 2.5–12; 4–60°C
Storage	Store at 4–8°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/interfering compounds. The quantity of microparticles may therefore require optimization for your application. We recommend the use of excess phosphopeptide-containing sample in order to saturate the microparticles.

NOTE: All reagents should be freshly prepared and of analytical grade to ensure optimal performance. The procedures, methods and buffer solutions serve as an example and are not intended to be limiting. MagReSyn® ZrO_2 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® ZrO_2

MagReSyn® ZrO_2 is supplied as a 50 $mg.ml^{-1}$ suspension in 20% ethanol. The shipping solution needs to be removed and the microparticles washed and equilibrated before use. The recommended protocol may be scaled up or down to suit your requirements. A minimum volume of 10 µl microparticle suspension is required per reaction to ensure a suitable pellet size for the aspiration of buffers. The current protocol is sufficient for the purification of phosphopeptides from ~500 µg of total protein digest.

1) Resuspend MagReSyn® ZrO_2 thoroughly by vortex mixing or inversion to ensure a homogenous suspension.
2) Transfer 40 µl (2 mg) MagReSyn® ZrO_2 to a 2 ml micro-centrifuge tube. *NOTE: 2 ml microcentrifuge tubes provide better mixing and agitation of microparticles during vortexing or end-over-end mixing than 1.5 ml tubes.*
3) Place the tube on a 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 the microparticles in 200 µl of 70% ethanol with gentle agitation (e.g. vortex mixing) for 5 min.
6) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the ethanol solution by aspiration with a pipette and discard.
7) Repeat steps 5 and 6.
8) Wash the microparticles in 100 µl of 1% NH_4OH with gentle agitation (e.g. vortex mixing) for 10 min.
9) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the buffer by aspiration with a pipette and discard.
10) Equilibrate the microparticles in 50 µl of loading buffer (1 M glycolic acid in 80% ACN, 5% TFA), allow 60 s for equilibration.
11) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the loading buffer by aspiration with a pipette.
12) Repeat steps 10 and 11 a further two times for a total of three equilibrations.
13) After removal of the loading buffer, MagReSyn® ZrO_2 is ready for binding of target phosphopeptides.

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

2.2. Phosphopeptide Enrichment Procedure

1) Adjust protein digest (containing ~500 µg total protein) with 1 equivalent volume of loading buffer (refer to **Section 4**) to the equilibrated MagReSyn® ZrO_2 microparticle pellet from **2.1**. *NOTE: if you have a dried peptide digest, resuspend the digest in a minimum of 100 µl of loading buffer before adding to the microparticle pellet.*
2) Resuspend the microparticles in the sample by vortexing or pipette aspiration.
3) Incubate for 20 min at room temperature with continuous mixing (e.g. slow vortexing) to ensure adequate sample and microparticle interaction.
4) Place the tube on the magnetic separator and allow the microparticles to clear. Remove and discard the coupling supernatant by aspiration with a pipette.
5) Remove unbound sample by washing with 100 µl of loading buffer (refer to **Section 4**) for 30 s with gentle agitation.
6) Place the tube on a magnetic separator and allow 10 s for the microparticles to clear. Remove the supernatant and discard.
7) Remove non-specifically bound peptides by resuspending the microparticles in 100 µl of wash buffer (aqueous solution of 80% ACN and 1% TFA) for 2 min with gentle agitation.
8) Place the tube on a magnetic separator and allow 10 s for the microparticles to clear. Remove the supernatant and discard.
9) Repeat steps 7 and 8 a further two times for a total of three washes.
10) Perform additional two washes using aqueous solution of 10% ACN and 0.2% TFA with magnetic recovery as above.
11) Elute the bound phosphopeptides from the microparticles by adding 80 µl elution buffer (1% NH_4OH) for 15 min. Ensure that the microparticles remain in suspension by constant gentle agitation during the elution step.
12) Repeat step 7 and 9 a further two times for a final elution volume of 240 µl. For a more concentrated elution, a minimum volume of 3 x 40 µl may be used for the elution procedure.
13) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the eluate containing the phosphopeptides and transfer to a new tube.
14) Add 60 µl of 10% Formic Acid (FA) to the 240 µl eluate, to acidify the solution.
15) Analyze the sample by mass spectrometry. It is recommended to desalt (e.g. C18) and concentrate the samples (e.g. by vacuum centrifugation or lyophilization) prior to MS analysis.

3. Recommended Storage

MagReSyn® ZrO_2 is supplied as a suspension of 50 $mg.ml^{-1}$ 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: 1 M glycolic acid in 80% acetonitrile (ACN) and 5% trifluoroacetic acid (TFA)
Wash buffer: 80% ACN, 1% 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	Incorrect binding pH	Ensure pH of loading buffer is between pH 2.5–3.0
	Insufficient reaction time	Extend the sample-microparticle incubation time to 30 min
	Interfering compounds in sample prevent binding	Desalt or dialyze sample into recommended binding buffer to remove potential interfering components
	Insufficient particle quantity	Increase quantity of MagReSyn® ZrO_2
Non-specific binding of peptides	Insufficient wash volume	Increase volume of wash buffer and ensure proper mixing
	Insufficient wash time	Increase time for each wash step to improve removal of non-specifically bound peptides.
	Incorrectly prepared buffers	Check calculations and prepare fresh buffers required for phosphopeptide enrichment.
Low recovery/ signal of phosphopeptides	Incorrect concentration of elution buffer	Ensure concentration of NH_4OH is above 0.5% - increase concentration and evaluate elution of phosphopeptides by MS
	Sample concentration too dilute	Concentrate samples via lyophilization or vacuum centrifugation prior to MS analysis.
	Ion suppression due to contaminants in the sample	Desalt phosphopeptides by HPLC (e.g. C18 RP-trap column or similar) or using solid-phase extraction (OLIGO®-R3 by Life Technologies or equivalent)

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