MagReSyn® Trypsin

Trypsin immobilized on magnetic microparticles

Ordering Information	
Cat. No.	**Quantity**
MR-TRP002	2 ml
MR-TRP005	5 ml
MR-TRP010	2 x 5 ml

For research use only

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1. Product Description

1.1. Overview

MagReSyn® Trypsin contains sequencing grade trypsin immobilized on a proprietary magnetic polymeric microparticle support. The ReSyn microparticle technology is differentiated from conventional solid or cracked bead technologies in that it provides a hyper-porous polymer network, which allows penetration of proteins throughout the volume of the microparticle. This enables multi-point covalent attachment of proteins such as trypsin, stabilizing the enzyme for application in non-standard conditions and preventing autolysis. The high content microparticles also allow for fast and efficient protein digestion.

1.2. MagReSyn® Technology Advantages

MagReSyn® Trypsin has been engineered to improve the quality of experimental data generated by mass spectrometry analysis of proteolytically digested samples. Magnetic microparticles allow the automation of sample digestions on magnetic bead handling stations, increasing throughput and improving experimental consistency. A key feature of immobilized proteolytic enzymes is the possibility of increased depth of sample coverage, as the enzymes can be removed prior to MS analysis. Immobilization enhances enzyme stability under non-standard experimental conditions such as the presence of organic solvents and chaotropic agents, thereby increasing the versatility of your proteomics workflow. The high trypsin content of the microparticles reduces protocol times as the recommended digestion period is only 1 h, achieving data quality usually only attainable with 24 h tryptic digestions. In addition, the compressibility of the microparticles reduces the interstitial spaces between the microparticles during washing and elution, leading to increased efficiency of these steps. MagReSyn® microparticles are separated rapidly (<10 s) using a standard magnetic separator, in comparison to alternative competitor microparticles that may take up to 4 min to clear.

MagReSyn® Technology Advantages	End-user Benefits
High trypsin content	Rapid digestion (≤60 min) Increased digestion efficiency for difficult-to-digest proteins Miniaturization of experiments Reduced sample volumes
Digestion in presence of denaturants (e.g. urea)	Increased digestion efficiency for difficult-to-digest proteins
Reduced trypsin autolysis	Proteolytic enzymes are removed from your samples resulting in improved data quality and reproducibility
Rapid magnetic separation	Simple and efficient removal of trypsin from digests High-throughput compatibility Automation of digestion possible if using magnetic bead handling station
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 digestion, proteomics, mass spectrometry sample preparation
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Functionality	Trypsin
Size (approx.)	5–10 µM
Formulation	15 $mg.ml^{-1}$ suspension in 50 mM acetic acid
Optimal activity	pH 8.0; 37ºC
Storage	Store at 4–8ºC until expiry date on label **DO NOT FREEZE**

1.4. Additional Equipment and Materials

Magnetic separator, Vortex mixer, Buffers and solutions

2. Sample preparation, MagReSyn® Trypsin equilibration and digestion

Several factors affect the efficiency of tryptic digestion. These include sample preparation, buffer composition and pH, presence of contaminants or interfering compounds, and the digestion temperature. MagReSyn® Trypsin allows for enhanced coverage and improves cleavage of difficult-to-digest proteins. If optimal performance is not achieved, refer to the recommended sample preparation/equilibration/digestion procedures listed below, as well as the Troubleshooting Guide (section 5).

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.

2.1. Sample Preparation

To ensure optimal digestion, target proteins require denaturation, reduction and alkylation.

1) Resuspend samples in 50 mM Tris pH 8.0 containing 6–8 M urea.
2) Add dithiothreitol (DTT) to a final concentration of 10 mM.
3) Incubate samples at room temperature for 1 h.
4) Add iodoacetamide (IAA) to a final concentration of 30 mM.
5) Incubate samples for 45 min in the dark.
6) Dilute or dialyze samples to ≤1 M urea using 50 mM Tris pH 8.

NOTE: Immobilized enzymes are stabilised against denaturation and the enzyme may be suitable for use under non-standard conditions such as increased concentration of chaotropic salts (albeit with reduced activity).

2.2. MagReSyn® Trypsin Equilibration

MagReSyn® Trypsin is supplied as a 15 $mg.ml^{-1}$ suspension in 50 mM acetic acid. The shipping solution needs to be removed and the microparticles equilibrated in digestion buffer (50 mM Tris pH 8.0) before use. Equilibrate sufficient quantities of microparticles for your digestion reactions as outlined below:

1) Resuspend MagReSyn® Trypsin by vortexing or inversion to ensure a homogeneous suspension.
2) Transfer 20 µl MagReSyn® Trypsin to a new tube (20 µl MagReSyn® Trypsin is sufficient to digest ~50 µg total protein in 50 mM Tris pH 8.0 in 60 min at 37°C).

NOTES: (1) The quantity of microparticles may be reduced to a minimum of 5 µl for starting protein quantities of less than 50 µg, using the equivalent ratio of microparticles to protein as outlined above. (2) For digestion under denaturing conditions (i.e. 2-6M urea or up to 0.5% SDS) increase microparticle amount to a minimum of 60 µl and the digestion time to a minimum of 2h at 37°C

3) Place the tube on magnetic separator and allow microparticles to clear.
4) Discard the storage solution by aspirating the supernatant with a pipette.
5) Equilibrate MagReSyn® Trypsin by gentle pipette resuspension in 50 µl of 50 mM Tris, pH 8.0.
6) Recover the microparticles by placing the tube on a magnetic separator and allow the microparticles to clear for 10 s.
7) Discard equilibration solution by aspirating the supernatant with a pipette.

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8) Repeat wash steps 5–7.
9) After removal of the equilibration buffer, MagReSyn® Trypsin is ready for protein digestion.

2.3. Protein Digestion Procedure

1) Add protein sample pre-equilibrated in 50 mM Tris pH 8.0 to MagReSyn® Trypsin from **2.2**.
2) Adjust the total reaction volume to 50 µl using 50 mM Tris.
3) Incubate sample for 60 min at 37˚C on a vortex or suitable microcentrifuge tube mixer to ensure the MagReSyn® Trypsin microparticles remain in suspension during digestion. Mixing by inversion is not recommended due to the low sample volumes.
4) Recover the microparticles by magnetic recovery as above.
5) Transfer supernatant (digested peptides) to a new microcentrifuge tube.
6) Add 50 µl 5 M NH_4OH to the microparticles.
7) Incubate sample for 20 min at RT on a vortex or suitable microcentrifuge tube mixer to ensure the MagReSyn® Trypsin microparticles remain in suspension during elution. Mixing by inversion is not recommended due to the low sample volumes.
8) Place the tube on the magnetic separator and allow the microparticles to clear.
9) Transfer the supernatant (eluted peptides) to the microcentrifuge tube containing remainder of digested peptides (step 2.3.5).
10) Add formic acid to the pooled peptides to a final concentration of 0.5%.

NOTE: When using low quantities of starting protein (less than 20 µg) we recommend reducing the volume of the digested proteins pre-MS by lyophilization or vacuum drying.

11) Desalt samples using C18 StageTips or ZipTip®, or suitable alternative desalting methods.
12) Proceed with mass spectrometric analysis.

NOTE: The efficiency of protein digestion may be assessed using SDS-PAGE should your protein quantity allow for it.

3. Recommended Storage

MagReSyn® Trypsin is supplied as a 15 $mg.ml^{-1}$ suspension in 50 mM acetic acid, 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 50 mM Acetic acid 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
Incomplete digestion	Incorrect digestion pH	Ensure recommended digestion pH of 8.0, check calibration of pH electrode
	Digestion temperature	Ensure digestion temperature is 37°C
	Digestion sites on target protein are not accessible	Digest at higher temperatures (up to 55°C) or evaluate digestion in elevated levels of chaotropic agents, e.g. 2–5 M urea
	Interfering compounds in starting protein sample	Desalt or dialyze sample into recommended digestion buffer to remove media components or contaminants
	Incorrect quantity of starting protein	Investigate alternative protein quantification methods
	Insufficient digestion time	Increase digestion time. Digest time is protein and complexity dependent i.e. complex lysates recommended digestion time is 4-6 hrs
Low sequence coverage or low mass spectrometry signal	Interfering compounds in final peptide solution	Desalt peptides post digestion using suitable desalting protocols (see **2.3.11**)
	Incorrect microparticle to protein ratio	Ensure that the recommended ratio of 30 µl microparticles to 50 µg protein is used. The protocol may be adjusted to a minimum of 5 µl microparticles, which can digest ~10 µg of protein in 1 h.

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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