



MagReSyn® Chymotrypsin

Chymotrypsin immobilized on magnetic microparticles

Ordering Information	
Cat. No.	Quantity
MR-CHY002	2 ml
MR-CHY005	5 ml
MR-CHY010	2 x 5 ml

For research use only

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1. Product Description

1.1. Overview

MagReSyn® Chymotrypsin is a proprietary magnetic polymeric microsphere support with immobilized chymotrypsin. The ReSyn microsphere technology is differentiated from conventional solid or cracked bead technologies by providing a loosely linked polymer network that allows penetration and multi-point attachment of chymotrypsin, thus stabilizing the enzyme and preventing autolysis. The high concentration of immobilized chymotrypsin allows for fast, efficient protein digestion.

1.2. MagReSyn® Technology Advantages

The exceptional digestion efficiency of MagReSyn® Chymotrypsin allows the miniaturization of experimental protocols by using small volumes of highly active functional microspheres, resulting in minimal reagent requirements and highly improved recovery of valuable biologicals in reduced volumes. In addition, the compressibility of the microspheres reduces the interstitial spaces between the microspheres during washing and elution, leading to increased efficiencies of these steps. MagReSyn® microspheres are separated rapidly (<10 sec) using a standard magnetic separator, in comparison to alternative competitor microspheres that may take up to 4 min to clear. The strong magnetic property minimizes costly sample loss by preventing accidental discarding/aspiration of the microspheres, thereby improving experimental reproducibility. The microspheres and buffers are engineered to deliver target proteins of exceptional purity to meet your stringent R&D requirements.

MagReSyn® Technology Advantages	End-user Benefits
High chymotrypsin content	Rapid digestion (≤ 30 min) Increased digestion efficiency of difficult-to-digest proteins Experimental miniaturization Reduced sample volumes
Digestion in the presence of denaturants (up to 8M urea)	Increased digestion efficiency of difficult-to-digest proteins
Reduced chymotrypsin autolysis	Proteolytic enzymes are removed from your samples resulting in improved data quality and reproducibility
Rapid magnetic separation	Simple and efficient removal of chymotrypsin 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 microspheres
Application	Protein digestion, Proteomics, Mass Spectrometry
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Functionality	Immobilized chymotrypsin
Size (approx.)	~5–10 μM
Formulation	10 mg.ml ⁻¹ suspension in 100 mM acetic acid
Optimal activity	pH 8.0; 25°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. Sample preparation, MagReSyn® Chymotrypsin equilibration and digestion procedures

Several factors affect the efficiency of chymotryptic digestion. These include sample preparation, buffer composition and pH, digestion temperature and the presence of contaminants or interfering compounds. MagReSyn® Chymotrypsin allows 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 6-8 M urea, 50 mM ammonium bicarbonate (or Tris) pH 8.
- 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 ammonium bicarbonate (or Tris) pH 8.

2.2. MagReSyn® Chymotrypsin Equilibration

MagReSyn® Chymotrypsin is supplied as a 10 mg.ml⁻¹ suspension in 100 mM acetic acid. The shipping solution needs to be removed and the microspheres equilibrated in digestion buffer (50 mM ammonium bicarbonate or Tris, pH 8) before use. Equilibrate sufficient aliquots of MagReSyn® Chymotrypsin for multiple digestion reactions as outlined below:

- 1) Resuspend MagReSyn® Chymotrypsin by vortexing or inversion to ensure a homogeneous suspension.
- 2) Transfer 20 μl MagReSyn® Chymotrypsin to a new tube (20 μl MagReSyn® Chymotrypsin is sufficient to digest ~50 μg total protein in 50 mM ammonium bicarbonate in 30 min at 37°C).

NOTES: (1) The quantity of microspheres may be reduced to a minimum of 5 μl for starting protein quantities of less than 50 μg, using the equivalent ratio of microspheres to protein as outlined above. (2) For digestion under denaturing conditions (i.e. 2-5M urea) increase microsphere amount to a minimum of 40 μl and the digestion time to a minimum of 2h at 37°C

- 3) Place the tube on a magnetic separator and allow microspheres to clear.
- 4) Discard the storage solution by aspirating the supernatant with a pipette.
- 5) Equilibrate MagReSyn® Chymotrypsin by gentle pipette resuspension in 50 μl of 50 mM ammonium bicarbonate or Tris.
- 6) Recover the microspheres by placing the tube on a magnetic separator and allow the microspheres to clear for 10 s.
- 7) Discard equilibration solution by aspirating the supernatant with a pipette.

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

2.3. Protein Digestion Procedure

- 1) Add protein sample equilibrated in 50 mM ammonium bicarbonate (or Tris) pH 8 to MagReSyn® Chymotrypsin from 2.2.
- 2) Adjust the total reaction volume to 50 µl using 50 mM ammonium bicarbonate (or Tris) .
- 3) Incubate sample for 30 min at 37°C on a vortex or microcentrifuge tube mixer to ensure that MagReSyn® Chymotrypsin microspheres remain in suspension. Do not mix the samples by inversion because of the low sample volumes.
- 4) Recover the microspheres by magnetic recovery as above.
- 5) Transfer supernatant (digested peptides) to a new microcentrifuge tube.
- 6) Add formic acid (FA) to a final concentration of 0.5% to the supernatant.

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

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

NOTE: The efficiency of the protein digestion procedure can be assessed using gel electrophoresis (SDS-PAGE).

3. Recommended Storage

MagReSyn® Chymotrypsin is supplied as a 10 mg.ml⁻¹ suspension in 100 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 microspheres, bacterial contamination, or centrifugal recovery may result in irreversible loss of capacity/performance. Resuspend well by vortex mixing before use.

4. General Information

Contact us at info@resynbio.com for larger microsphere quantities or customized microsphere 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 100 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 digestion pH is 8.0
	Incorrect digestion temperature	Digestion should be carried out at 37°C
	Insufficient digest time	Increase digestion time. Digest time is protein and complexity dependent i.e. complex lysates recommended digestion time is 2-3 hrs
	Digestion sites on target protein not accessible	Digest at higher temperatures (up to 55°C) or evaluate digestion in elevated levels of chaotropic agents, e.g. 2–5M urea
	Interfering compounds in starting protein sample	Desalt or dialyze sample into recommended digestion buffer to remove media components or other contaminants
	Incorrect starting protein quantity	Investigate alternative protein quantification methods
Low sequence coverage or low mass spectrometry signal	Insufficient peptide recovery	Perform a microsphere wash step once digestion is complete (section 2.3, step 4)
	Interfering compounds in final peptide solution	Desalt peptides post digestion using suitable desalting protocols (section 2.3, step 7)
	Incorrect microsphere to protein ratio	Ensure that the recommended ratio of 20 µl microspheres to 50 µg protein is used. The protocol may be adjusted to a minimum of 5 µl microspheres, capable of digesting ~10 µg of protein

Please contact our customer care number (+2712 841 4153) for more information or e-mail info@resynbio.com should your specific problem not be addressed in our troubleshooting guide.