



## MagReSyn® Ti-IMAC : Reactivation/Chelation Protocol

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

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#### 1. Overview

This protocol is intended to serve as a guideline for the re-activation of MagReSyn® Ti-IMAC microparticles through chelation of titanium (Ti<sup>4+</sup>) ions. MagReSyn® Ti-IMAC microparticles consist of titanium ions (Ti<sup>4+</sup>) chelated to phosphate-functional MagReSyn® polymer microparticles. The relative instability of chelated titanium ions unfortunately results in a relatively short half-life of 6 months for the product. We therefore provide this protocol for re-activation as a courtesy to customers whose applications may require use of extended periods. **NOTE:** We recommend that you use the product supplied by ReSyn Biosciences within the stated expiry date since these are subject to our stringent QC processes. Reactivated microparticles cannot be guaranteed by ReSyn Biosciences.

#### 2. Important Notes

The procedures, methods and buffer solutions described below serve as a guideline only. All reagents and buffers should be freshly prepared and of analytical grade to ensure optimal performance. Factors that may affect the efficiency of the reactivation procedure include pH, buffer composition, and the presence of low abundance contaminants/interfering compounds. We recommend the use of excess reactivation solution in order to saturate the microparticles with titanium ions. The microparticles should be resuspended to the same volume after the reactivation procedure, such that the formulation (mg microparticles per ml solution) does not change from the original product formulation, ensuring compatibility with our phosphopeptide enrichment protocols.

#### 3. Buffers, Chemicals and Solutions

- 10% Hydrochloric acid (HCl)
- 1 M Tris buffer pH 9.0
- Titanium(IV) chloride solution (Sigma Aldrich Cat# 404985)
- MS-grade ultrapure water
- 20% ethanol

#### 4. Reactivation Procedure

##### 4.1. microparticle Equilibration

MagReSyn® Ti-IMAC is supplied as a 20 mg.ml<sup>-1</sup> suspension in 20% ethanol. The shipping solution needs to be removed and the microparticles washed and equilibrated before reactivation. The recommended protocol may be scaled up or down to suit your requirements.

- 1) Resuspend MagReSyn® Ti-IMAC thoroughly by vortex mixing or inversion to ensure a homogenous suspension.
- 2) Transfer 1 ml (20 mg) MagReSyn® Ti-IMAC to a 2 ml microcentrifuge tube. **NOTE:** 2 ml microcentrifuge tubes provide better mixing of microparticles during vortexing or end-over-end mixing than 1.5 ml tubes.
- 3) Place the tube on a magnetic separator and allow microparticles to clear.
- 4) Remove the shipping solution by aspiration with a pipette and discard.
- 5) Wash the microparticles in 500 µl of 10% HCl with gentle agitation for 30 sec (e.g. vortex mixing at slow speed or end over end mixing).
- 6) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the 10% HCl solution by aspiration with a pipette and discard.

- 7) Wash the microparticles in 1 ml of MS-grade water with gentle agitation (e.g. vortex mixing) for 30 s.
- 8) Place the tube on the magnetic separator and allow the microparticles to clear. Remove the water by aspiration with a pipette and discard.
- 9) Repeat steps 7-8 and resuspend the microparticles in 1 ml of MS-grade water. Microparticles are suitable for reactivation.

#### 4.2. Titanium(IV) chloride preparation

Titanium(IV) chloride is supplied as a solution of 0.09 M in 20% HCl. The chemical needs to be diluted and adjusted to pH 2.0 with 1 M Tris buffer pH 9.0 prior to chelation to the MagReSyn® Ti-IMAC microparticles. **NOTE:** Extreme care is required during pH adjustment, the formation of a white precipitate is indicative of incorrect pH or presence of impurities. Precipitated solutions should be discarded and a new activation solution prepared.

- 1) Use a clean glass syringe to transfer 1 ml of Ti(IV) chloride stock solution to a new 15 ml conical tube.
- 2) Adjust pH by dropwise addition of 1 M Tris pH 9.0, mix by regular vortexing and monitor the pH using a clean glass pH probe. **NOTE:** Precipitated solution should not be used for activation.
- 3) Adjust the final volume to 10 ml with MS-grade water. Check the solution to ensure that it is free from precipitate. The Ti(IV) chloride solution is now ready for chelation to the equilibrated microparticles from 4.1

#### 4.3. Titanium(IV) chloride chelation

- 1) Magnetically recover the equilibrated microparticles (4.1, step 9) and discard the supernatant.
- 2) Add 1 ml of Ti(IV) chloride pH 2.0 (4.2, step 3) to the microparticles and place on an end-over end mixer for 16 h at room temperature.
- 3) Place the tube on a magnetic separator and allow the microparticles to clear.
- 4) Remove the spent Ti(IV) chloride solution by aspiration with a pipette and discard.
- 5) Wash the microparticles in 3 x 1 ml of 20% ethanol with gentle agitation (e.g. vortex mixing) for 10 sec in between washes.
- 6) Resuspend the microparticles in 20% ethanol to a total volume of 1 ml. The microparticles can now be utilized for phosphopeptide enrichment.

#### 5. General Information & Disclaimers

Visit our website ([www.resynbio.com](http://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) of the product (available at [www.resynbio.com](http://www.resynbio.com)) as well as the other chemicals required for using the product. Chemical reagents, buffers and biological material 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 and legal information.

#### 6. Troubleshooting Guide

Identified Problem	Possible Cause	Suggested Remedy
Titanium(IV) chloride solution precipitates out of solution during pH adjustment	pH too high	Ensure pH of solution does not exceed pH 2.5
	Impurities in chemicals or solutions	Ensure that all chemicals used are of analytical grade and that the water used is MS-grade
	pH probe not clean	Ensure that the pH probe used for pH adjustment is rinsed thoroughly.

**Please contact us via e-mail at [info@resynbio.com](mailto:info@resynbio.com) should your specific problem not be addressed in our troubleshooting guide.**