ReSyn Biosciences

MagReSyn® NTA Screening Kit

Metal ion affinity magnetic microparticles

Ordering Information	
Cat. #	Quantity
MR-NTA-KIT	4 x 0.5 ml

This product is for research use only

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1. Product Description

1.1. Overview

MagReSyn® NTA-KIT is a screening kit consisting of a proprietary magnetic polymeric microparticle support chelated with various divalent metal ions (Ni^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}) for affinity capture, purification and recovery of polyhistidine-tagged fusion proteins. Since different types of His-tagged proteins have varying affinities for a particular metal ion, MagReSyn® NTA-KIT provides an effective means to identify the most suitable metal ion for optimal purification of your particular His-tagged protein. The ReSyn technology is differentiated from conventional polymer technologies in that it comprises a hyper-porous polymer network that allows penetration and binding of biomolecules throughout the volume of the microparticle, leading to an increased capacity for the binding of biological molecules. The high functional group density allows for multi-point affinity capture of histidine-tagged biomolecules, resulting in stronger binding of the target proteins. This feature enables the use of increased imidazole concentrations in the binding buffer, thereby reducing non-specific binding of contaminating proteins, concomitantly resulting in higher purity of your target proteins. MagReSyn® NTA does not require sample clarification (after cell disruption) prior to protein isolation, improving its application in automated purification protocols on magnetic bead-handling stations.

1.2. Advantages of MagReSyn® Technology

MagReSyn® NTA has been engineered to provide target His-tagged protein of exceptional purity. The compressibility of the microparticles reduces the interstitial spaces between the microparticles during washing and elution procedures, leading to increased efficiencies and recoveries. MagReSyn® microparticles are separated rapidly (<10 s) using a standard magnetic separator, in comparison to leading competitor microparticle technologies that may 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 buffers we have recommended are engineered to deliver maximum binding capacity and protein purity to meet your stringent R&D requirements.

MagReSyn® Technology Advantages	End-user Benefits
High specificity for 6x His-tagged proteins	High purity of target proteins (≥ 97%) Minimize/reduce additional purification steps and reagents
Compatible with 8M Urea	No sample clean-up/desalting required for urea-containing samples prior to binding
Rapid magnetic separation	Reduced particle carry-over Improved experimental reproducibility Rapid protocols
Resistant to oxidation (rust)	Reduced sample contamination Improved shelf-life

1.3. Product Information

Product Specifications	
Description	Iron oxide-containing magnetic polymer microparticles
Application	Immobilized Metal Ion Affinity Chromatography (IMAC) of Histidine (His)-tagged proteins
Matrix	Proprietary polymer
Core	Iron (II, III) oxide (Magnetite)
Functional group	Nitrilotriacetic acid (NTA) with chelated nickel (Ni^{2+}), Copper (Cu^{2+}), Cobalt (Co^{2+}) and Zinc (Zn^{2+})
Binding capacity	≥ 1.0 mg of a His-tagged GFP.ml^{-1} suspension
Particle Size	~5–10 µM
Formulation	2.5%: 25 mg.ml^{-1} suspension in 20% ethanol
Stability	pH 3.5–10; 4–60°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. Binding and Elution Procedure

Several factors may affect the efficiency of metal ion affinity purification of 6x His-tagged proteins. These include the type of metal ion, buffer composition and pH, presence of contaminants/interfering compounds, presence of proteases that can degrade the target protein and the location of the 6x His-tag on the protein (e.g. N-terminal, C-terminal or sandwich fusion tag). MagReSyn® NTA products are compatible with 8M urea in binding/washing and elution buffers. If optimal performance is not achieved, please refer to the recommended binding/washing/elution procedures in this guide, as well as the Troubleshooting Guide (section 6).

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® NTA is compatible with a range of different buffers for binding/adsorption and elution/desorption. Achievable purity and yield are ligand dependent and experimental conditions should be optimized for each ligand purified.

2.1. MagReSyn® NTA Equilibration

Each of the 4 products in the MagReSyn® NTA-KIT is supplied as 0.5 ml of a 25 mg.ml^{-1} suspension in 20% ethanol. The shipping solution needs to be removed and the microparticles equilibrated in binding buffer (e.g. 80 mM sodium phosphate pH 7.4–8.0, 40 mM imidazole, 1.0 M NaCl) before use. The recommended protocol can be scaled up or down to suit your requirements - the current protocol is estimated for binding ~20 µg of histidine-tagged protein.

1) Resuspend each MagReSyn® NTA-KIT product thoroughly by vortex mixing for 3 s to ensure a homogenous suspension.
2) Transfer 20 µl (sufficient to bind ~20 µg of histidine-tagged protein) of each MagReSyn® NTA-KIT product to a new tube.
3) Place the tubes on the magnetic separator and allow the microparticles to clear.
4) Remove the shipping solution by aspiration with a pipette and discard.
5) Wash/equilibrate the microparticles for 30 s in 200 µl binding buffer.
6) Place the tubes on the magnetic separator and allow the microparticles to clear. Invert the magnet twice with the tube in place to collect any microparticles remaining in the cap.
7) Remove the binding buffer by aspiration with a pipette and repeat steps 5 and 6 twice for a total of three washes.
8) After aspiration of the binding buffer from step 5, MagReSyn® NTA is ready for binding of 6x His-tagged proteins.

2.2. Protein binding procedure

As a standard measure it is recommended that your protein-containing samples be clarified by filtration through a 0.2 µm filter or centrifuged at 10,000 x g for 5 min prior to protein purification. For automated applications the removal of this step should be validated as necessary.

1) Add the sample containing His-tagged protein to each equilibrated MagReSyn® NTA from **2.1**. Adjust the binding volume by dilution with at least 1 volume of binding buffer (refer to **2.1**) and mix thoroughly by vortexing for 3 s

2) Allow proteins to bind to the microparticles for 5 min at room temperature with gentle intermittent agitation.
3) Place the tubes on the magnetic separator and allow the microparticles to clear.
4) Aspirate the supernatant with a pipette. The supernatant can either be discarded, or subsequently used for protein quantification or electrophoresis (e.g. to ascertain unbound protein).
5) Wash the bound protein by resuspension in 200 µl binding buffer, wash for 30 s, vortexing intermittently.
6) Place the tubes on the magnetic separator and allow the microparticles to clear. In the event that there are microparticles in the cap of the tube, invert the magnetic separator with the tube in place to collect the microparticles.
7) Aspirate the wash buffer with a pipette.
8) Repeat steps 5–7 twice for a total of three washes. The supernatants from the wash steps can either be discarded or pooled for protein quantification or protein tracking.

2.3. Protein Elution Procedure

1) Add 20–50 µl of elution buffer (80 mM sodium phosphate pH 7.4–8.0, 500 mM NaCl, 500 mM imidazole) to the microparticle pellet from **2.2**. Mix well by pipetting or vortexing.
2) Allow the proteins to elute for 2 min at room temperature.
3) Place the tubes on the magnetic separator and allow the microparticles to clear. Remove the eluate containing the protein of interest by aspiration with a pipette.
4) To improve recovery of his-tagged proteins repeat steps 1–3 a further two times with an additional 20–50 µl elution buffer (three eluates in total). Combine/pool the three eluates. The protein is now ready for further experimentation or analysis.

2.4. Effect of Imidazole and NaCl on Protein Binding and Elution

The optimal imidazole concentration required for efficient purification will depend on the target protein and the location of the histidine tag (e.g. N-terminal, C-terminal or fusion). Lower imidazole concentrations can be used to promote recovery/yield, albeit potentially at the expense of purity. MagReSyn® microparticles are formulated to bind 6x His-tagged proteins in the presence of up to 80 mM imidazole without notably affecting target protein recovery or yield (protein and tag dependent). While increasing the imidazole concentration in the binding/washing steps may be used to increase the purity of the target protein, this may lead to a reduction in yield. For most proteins, 500 mM imidazole is usually sufficient for elution, while other proteins may require up to 1 M imidazole for efficient elution. Be sure to check the compatibility of your protein's function with these conditions before proceeding with high imidazole elution. The concentration of the NaCl may be increased up to 2 M to reduce non-specific ionic interactions, potentially increasing the purity of the tagged proteins. It is recommended to load the microparticles with an excess of His-tagged protein since this may assist in minimizing non-specific interactions, maximizing the purity of your target protein..

3. Recommended Storage

MagReSyn® NTA-KIT contains 4 x 0.5 ml products supplied as suspensions of 25 mg.ml^{-1} in 20% ethanol and should be stored at 2–8^{o}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. Reagent/Chemical Compatibility

MagReSyn® NTA is compatible with samples containing the following buffer components:

Reagent	Concentration
Urea	≤8 M
Triton® X-100	≤5%
Tween® 20	≤1%
Tris, MOPS, Sodium/Potassium phosphate	≤100 mM
NaCl	≤2 M
Glycerol	≤50%

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
Proteins do not bind to the microparticles as expected	Incorrect binding pH	Increase pH of binding buffer to at least pH 7.4
	Protein of interest degraded	Add protease inhibitors to crude protein extract
	Interfering compounds in sample prevent binding	Desalt or dialyze sample into recommended binding buffer to remove media components or other interfering contaminants
	Insufficient quantity of particles	Increase amount of MagReSyn® NTA microparticles
	Protein content too low	Increase protein content by sample concentration or increasing quantity of starting material
	Incorrect protein sequence	Confirm clone by sequencing
Low recovery of proteins during elution	Affinity binding very strong	Increase imidazole concentration in elution buffer
	Protein may have metal-binding domain	Increase imidazole concentration or elute with acidic buffer, e.g. citrate pH 3–4
	Protein degradation occurs during purification	Add protease inhibitors to samples and buffers to prevent proteolytic degradation. Use freshly prepared samples and solutions, reduce sample preparation times where possible, work at 4^{o}C
	Protein may be unstable or inactive in elution buffer	Determine optimal pH and salt stability of protein of interest, adapt protocol accordingly
Insufficient purity of eluted protein (co-elution of contaminating protein)	Inefficient washing	Increase number and volumes of wash steps. Increase NaCl concentration of wash buffer (up to 2 M). Increase imidazole concentration in wash buffer up to 80 mM
	Specific degradation of target protein	Add protease inhibitors to extract and to binding/washing and elution buffers to prevent proteolysis
Protein of interest present in sample after binding	Insufficient microparticle quantity used	Increase amount of MagReSyn® NTA microparticles used for purification
Target protein inactive after elution	Interference by imidazole or buffer salts	Remove imidazole or buffer salts by dialysis, filtration, precipitation or size-exclusion chromatography. Decrease imidazole concentration for elution or elute in acidic buffer, e.g. citrate pH 3–4; immediately increase pH after elution with suitable basic buffer.
Target protein is incompatible with downstream applications	Interference by imidazole, buffers or salts	Remove imidazole by dialysis, filtration, precipitation or size-exclusion chromatography.

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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