MagReSyn® NTA: Increased purity of histidine-tagged proteins through enhanced binding affinity

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Abstract

The unique polymer structure of ReSyn microparticle technology provides an increased functional group density over alternate solid supports. This not only provides for a high capacity, but can further serve to improve the strength of molecular interactions, increasing the specificity of the microspheres for the target protein, and enabling new applications where increased binding strength is desirable. The application note compares the binding strength, and purity of his-tagged protein purified with MagReSyn® NTA, and an Alternate Magnetic NTA Product from a leading supplier.

Introduction

Immobilized metal ion affinity chromatography (IMAC) is a widely used method for protein isolation and purification. The purification is achieved through the affinity of a chelated divalent metal (usually Ni^{2+}) with a chain of histidine residues expressed in sequence in the recombinant protein. MagReSyn® NTA microparticles are pre-chelated with nickel (Ni^{2+}) ions and have been engineered for exceptional specificity; providing highly pure target proteins.

The microparticles were evaluated for the stepwise gradient elution of *6xHis*-tagged protein isolated from a crude *E.coli* lysate. The strength of the binding interaction is paramount to the purity and specificity that can be achieved since it allows for e.g. washing in increased imidazole concentrations, thereby increasing purity by removing histidine rich or non-specifically interacting proteins from the microparticles. The target *6xHis*-tagged protein can then be eluted at very high purity.

The increased affinity of MagReSyn® NTA can enable new applications. As an example, a publication by Moss *et al*., Analytical Biochemistry, 2015, Volume 484, pg. 75-81, describes the development of a simple and sensitive assay system for quantifying diagnostically relevant proteases. The publication found the performance of MagReSyn® NTA drastically improved the sensitivity and specificity of the protease assay under development for MMP2 and ADAM8. The authors demonstrated the assay could be used for detection of the proteases in urine, illustrating the potential for diagnostic application of the assay.

Materials

Buffers for MagReSyn® NTA

- *Binding/Wash Buffer*: 80 mM sodium phosphate pH 7.4, 40 mM imidazole, 1 M NaCl
- *Elution Buffer*: 80 mM sodium phosphate pH 7.4, 1 M NaCl, containing concentrations of either 40 mM, 80 mM, 100 mM, 150 mM, 250 mM, 500 mM or 1 M Imidazole.

Buffers for Alternate Supplier Magnetic NTA

- *Binding/Wash Buffer*: 80 mM sodium phosphate pH 7.4, 20 mM imidazole, 0.5 M NaCl
- *Elution Buffer*: 80 mM sodium phosphate pH 7.4, 0.5 M NaCl, containing concentrations of either 40 mM, 80 mM, 100 mM, 150 mM, 250 mM, 500 mM or 1 M Imidazole.

A magnetic separator was used to isolate magnetic beads between each step of the protocol.

Methods

Protein Production

E. coli cell culture (1 ml IPTG induced) producing a target *6xHis*-tagged was harvested by centrifugation (10,000 x g for 5 min). The cell pellet was washed in *PBS* (80 mM sodium phosphate pH 7.4 with 150 mM NaCl; 2 x 500 µl washes) to remove medium components. The cells were lysed in 1 ml PBS using sonication (Bandelin Sonopuls, 70% power, 2 x 5 min on ice). The solution was clarified by centrifugation (10,000 x g for 5 min). The supernatant containing *6xHis*-tagged protein was removed and 100 µl aliquots were diluted with various preparations of double strength *Binding Buffer* to obtain final concentrations of the buffer components recommended by the suppliers (listed above). The sample was subsequently applied to equilibrated MagReSyn® NTA or Alternate Supplier's microparticles.

Equilibration of Microparticles

Microparticles were equilibrated according to the manufacturer's protocol. Briefly: MagReSyn® NTA is supplied as a 25 $mg.ml^{-1}$ suspension in 20% ethanol. The shipping solution was removed and the microparticles equilibrated in *Binding/Wash Buffer* prior to use. The microparticles were recovered by application to a magnetic separator between each step. The microparticles were thoroughly resuspended by vortex mixing for 3 seconds, and comparative aliquots of MagReSyn® NTA (10 µl) or Alternate Supplier (10 µl) were removed for equilibration. The microparticles were pipetted into a 2 ml micro-centrifuge tube, and recovered by application to a magnetic separator. The shipping solution was removed by aspiration with a pipette and discarded, and the microparticles subsequently equilibrated in *Binding/Wash buffer* for three washes of 200 µl for 30 sec each.

Protein binding procedure

The sample containing *6xHis*-tagged protein was added to the equilibrated microparticle suspensions and mixed thoroughly by pipetting up and down. The protein-containing sample was allowed to interact with the microparticles for 15 min at room temperature with gentle agitation to ensure that the microparticles remained in suspension for the duration of the binding procedure. The tube was then placed on the magnetic separator, to capture the magnetic particles, and the supernatant aspirated with a pipette. The microparticles were washed three times by resuspension in 200 µl of the appropriate *Binding/Wash Buffer* for 30 sec each (to remove unbound protein), with recovery by capture on a magnetic separator. The wash fractions can either be discarded or retained for analysis.

Protein Elution Procedure

For comparison of purity, protein was bound and eluted using the recommended supplier's protocols. The leading alternate supplier of magnetic microparticles was selected for further comparison. To determine comparative binding strength, the enriched *6xHis*-tagged protein was eluted using sequential increases in imidazole concentration (40 mM to 1 M) from the microparticles using 10 µl of the elution buffer, and mixed well by pipetting. The protein was allowed to elute for 1 min at room temperature in each imidazole concentration, before placing the tube on the magnetic separator to capture the microparticles. The eluate was removed by aspiration with a pipette and transferred to a new tube for analysis by gel electrophoresis. To potentially improve recovery of *6xHis*-tagged proteins, the elution procedure was repeated with an additional 10 µl of each elution buffer (total of 20 µl), and pooled for analysis.

Gel Electrophoresis

The comparative purity and elution profile of the *6xHis*-tagged proteins were assessed by gel electrophoresis using NUPAGE® 4-12% Bis-Tris precast gels (Life Technologies) according to the manufacturer's protocol. For comparative purity, the protein bands were visualized by staining proteins for 16 h with Colloidal Coomassie Blue stain (Neuhoff *et al*, 1990, Electrophoresis, Vol 11, pg. 101-117). The gels were destained in ultrapure water. The gel images were captured on a Syngene G:BOX gel documentation system (automatic exposure) and protein quantity analysed by densitometry (GeneSnap software). The criterion for selecting the leading Alternate Product, for subsequent evaluation of binding strength (stepwise gradient elution), was purity of the target *6xHis*-tagged target protein.

Results & Discussion

The comparative purity of *6xHis*-tagged proteins eluted from magnetic NTA microparticles is illustrated in **Figure 1**. The leading supplier was selected and comparative binding strength illustrated by stepwise gradient imidazole elution (**Figure 2**). At the lowest imidazole concentration tested (40 mM), a comparatively low quantity of target protein was eluted from MagReSyn® NTA, while almost 25% of the protein was eluted from the Alternate supplier's product under the same conditions.

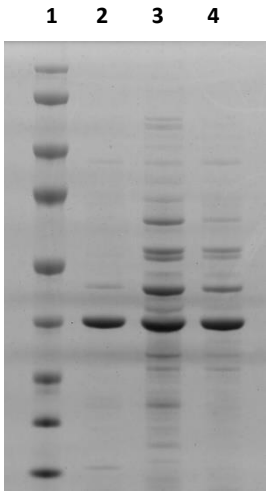
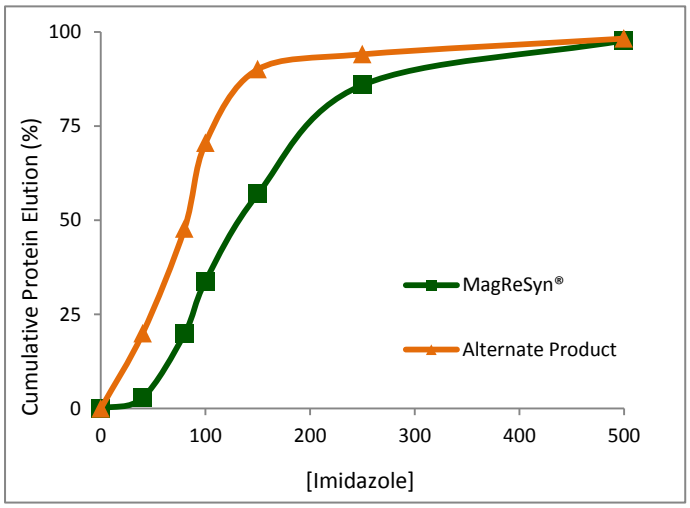


Figure 1 (left): Comparative purity of eluted *6xHis*-tagged target protein. The gel was loaded to obtain equivalent quantity of target protein. Lane 1: Molecular weight markers, Lane 2: MagReSyn® NTA, Lane 3 & 4: Alternate supplier products. Alternate Product in lane 4 was selected for further comparison.

Figure 2 (right): Elution profile of *6xHis*-tagged target protein using increasing concentrations of imidazole in the elution buffer, peak elution occurs at 150 to 250 mM imidazole for MagReSyn® NTA, while peak elution occurs at 80 mM for the Alternate Product from a leading supplier.



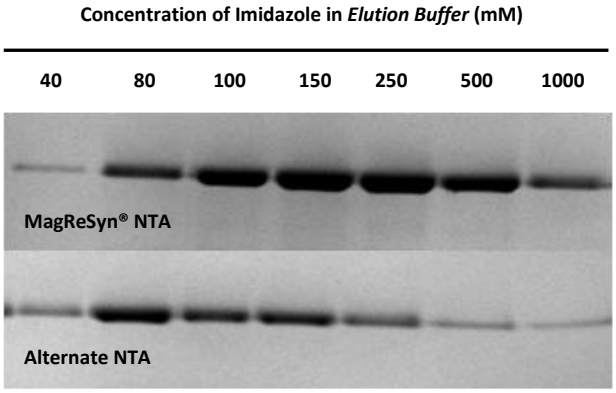


Figure 3 (left): Quantitative profile (densitometry) of stepwise elution for histidine tagged proteins from MagReSyn® NTA and leading Alternate product. Due to the increased capacity of MagReSyn® over the capacity of the Alternate magnetic NTA Product, the data was normalised for protein content (% of total protein eluted).

Acknowledgements

Thank you to Dr Michael Crampton at CSIR Biosciences (Pretoria, South Africa) for providing a sample of *E. coli* expressing a soluble *6xHis*-tagged protein.

Conclusions

MagReSyn® NTA's high functional group density and hyperporus polymer structure translates into an increased affinity for *6xHis*-tag proteins, demonstrated by comparative competitive elution against a leading Alternate Product. The increased affinity permits more stringent binding and washing conditions, providing improved purity of the target protein. The increased binding strength may enable and enhance research and diagnostic applications including on-bead analysis, quantification, and on-bead protein re-folding.

Ordering Information

Description	*Product Code*
MagReSyn® NTA 2 ml	**MR-NTA002**
MagReSyn® NTA 5 ml	**MR-NTA005**
MagReSyn® NTA 10 ml	**MR-NTA010**

Please contact info@resynbio.com should you have any queries relating to this application or product.

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