High-throughput screening for antibodies and histidine-tagged proteins using Mag Sepharose™ magnetic beads

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Mag Sepharose magnetic beads have been developed to simplify handling in protein sample preparation. The beads are an excellent choice for enrichment, small-scale purification, and screening of target proteins. In this study, automated screening methods for purification of monoclonal antibodies and histidine-tagged proteins are described. A combination of Mag Sepharose magnetic beads in a 96-well microplate, MagnaBot™ 96 Magnetic Separation Device and Tecan Freedom EVO™ liquid handling workstation was used.

Introduction

The Mag Sepharose platform combines well-established enrichment and purification methods with the magnetic bead format to provide high quality and reproducible results. The magnetic beads are scalable and provide simple capture of target protein in small or large sample volumes, from low microliter to high milliliter scale. Mag Sepharose magnetic beads are suitable for enrichment, small-scale purification and screening of target proteins such as in screening for optimal purification conditions.

Reproducible purification

Combining Tecan Freedom EVO, MagnaBot 96 Magnetic Separation Device, and Protein G Mag Sepharose Xtra ensures high reproducibility. To demonstrate this, 96 replicate runs on a 96-well microplate filled with Protein G Mag Sepharose Xtra beads were performed to purify a monoclonal human IgG expressed in CHO cells. The load was 60% of the total binding capacity and the yield of the eluted fractions was determined via absorbance measurements.

Figure 1 shows the yield of the monoclonal human IgG. The results show good well-to-well reproducibility with low relative standard deviation (RSD) of 4.6%. All 12 samples from wells H 1–12 in the 96-well microplate had a lower amount of eluted monoclonal human IgG. This reduced amount of eluted human IgG Indicates a systematic error but no further investigation was made. The relative standard deviation for 96 samples was only 3.0% when omitting the 12 samples from wells H 1-12.

Flexible purification

Adapting the purification scale to different sample volumes is one advantage with the magnetic bead format. In this study, different volumes of magnetic beads were scaled down from 5 µl magnetic beads to 1 µl. Human IgG was purified using Tecan Freedom EVO, MagnaBot 96 Magnetic Separation Device, and Protein G Mag Sepharose Xtra filled in 96-well plates. The yield and recovery of the eluted fractions was determined by absorbance measurements. The purification showed good well-to-well variation with relative standard deviations (Fig 2).

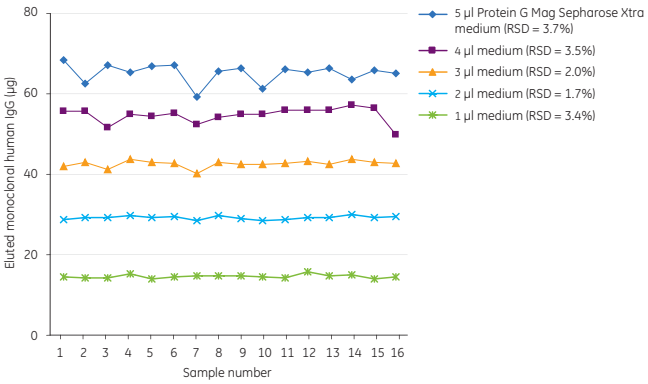

Fig 2. Human IgG was loaded with 50% of the total binding capacity for each magnetic bead volume. The yield and recovery of the eluted fractions was determined by absorbance measurements. The results showed good well-to-well variation with low relative standard deviations.

Screening for optimal IMAC purification conditions

Purification of histidine-tagged proteins by immobilized metal ion adsorption chromatography (IMAC) is a balance between yield and purity, modulated by the imidazole concentration in the sample and binding/wash buffer. The optimal imidazole concentration is protein dependent and can be determined for each histidine-tagged protein.

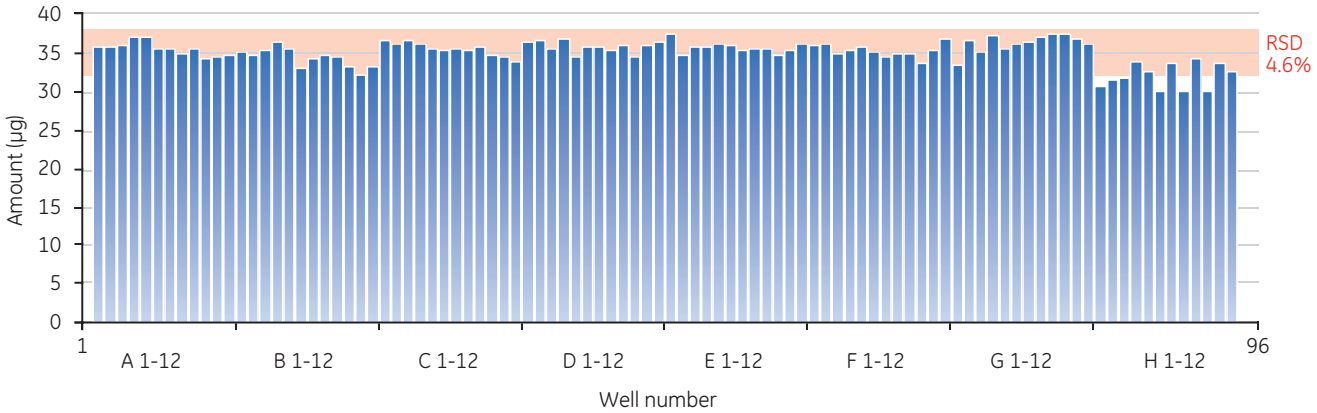

Fig 1. Elution reproducibility over 96 runs of monoclonal human IgG purified from CHO cells using Protein G Mag Sepharose Xtra magnetic beads.

A screening study for optimal sample loading and wash conditions was performed with eight different imidazole concentrations and four different sample loads, varying from 25% to 100% of the total binding capacity of His Mag Sepharose Ni. Histidine-tagged green fluorescent protein, GFP-$(His)_6$, was purified from *Escherichia coli* lysate using Tecan Freedom EVO, MagnaBot 96 Magnetic Separation Device, and His Mag Sepharose Ni filled in 96-well plates. The yield and purity of the eluted fractions was determined by absorbance measurements and SDS-PAGE analysis, respectively.

The results showed that during sample application and wash, a good balance between yield and purity was obtained with 40 mM imidazole (Fig 3) and a sample load of 50% to 100% of the total binding capacity (Fig 4).

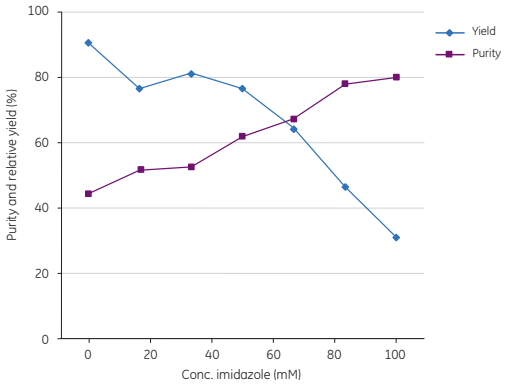

Fig 3. Purity and relative yield of GFP-$(His)_6$ with a 50% sample load relative to the total binding capacity of His Mag Sepharose Ni.

Conclusions

Mag Sepharose magnetic beads filled in 96-well plates and combined with robotics provide an excellent platform for screening of protein constructs and conditions that can be tested in drug development and structural studies.

Using screening methodology with Mag Sepharose beads combined with robotics enables highly reproducible results, as well as a fast and cost-effective screening with limited effort.

To see an animation demonstrating the practical use of His Mag Sepharose Ni beads, visit http://www.gelifesciences.com/aptrix/upp01077.nsf/content/sample_preparation~news~mag_sepharose?OpenDocument&intcmp=ibc000005

Ordering information

Product	Code number
Protein G Mag Sepharose Xtra, 2 × 1 ml	28-9670-66
Protein G Mag Sepharose Xtra, 5 × 1 ml	28-9670-70
His Mag Sepharose Ni, 2 × 1 ml	28-9673-88
His Mag Sepharose Ni, 5 × 1 ml	28-9673-90
His Mag Sepharose Ni, 10 × 1 ml	28-9799-17
MagRack 6	28-9489-64

For more information on the Mag Sepharose platform, visit www.gelifesciences.com/sampleprep

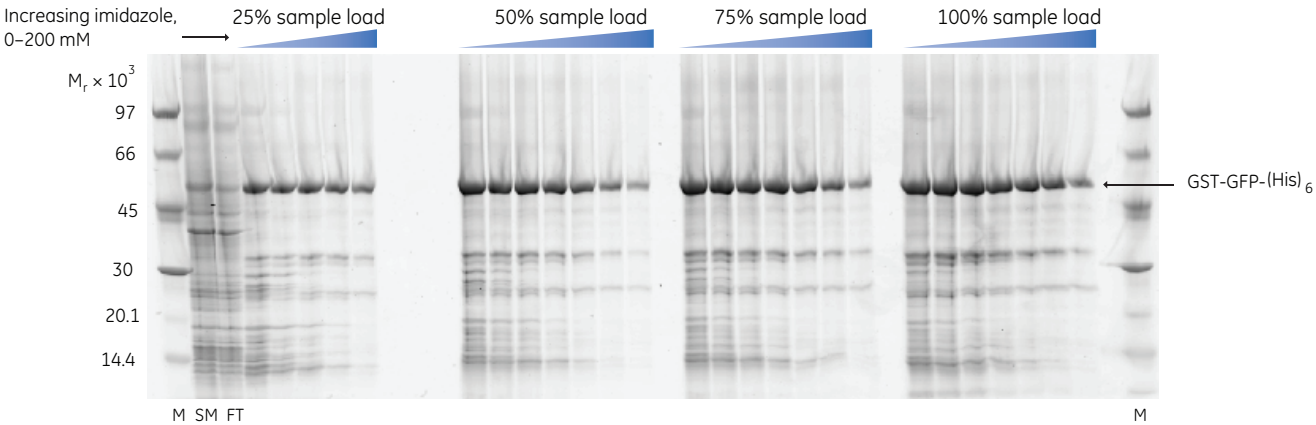

Fig 4. SDS-PAGE of GFP-$(His)_6$ enriched from a background of *E. coli* protein using His Mag Sepharose Ni beads. GFP-$(His)_6$ was detected using Deep Purple™ Total Protein Stain and Ettan™ DIGE Imager. The gel image was analyzed with ImageQuant™ TL software. M = molecular weight markers, SM = start material, FT = flowthrough from a sample load of 75% of the total binding capacity, 40 mM imidazole.