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High capacity magnetic supports for automated antibody and epitope-tagged protein purifications

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ABSTRACT

For academic, biotech, and pharmaceutical scientists who are screening clones or performing high throughput purifications, the goal is to automate the sample processing without sacrificing binding capacity. Traditionally, magnetic beads have facilitated process automation, but lack the high binding capacity of agarose resins. On the other hand, agarose or Sepharose™ resins have high binding capacity, but are not amenable to automation. Magnetic agarose combines the best of both formats, by enabling high throughput sample processing with high protein binding capacity. Here we demonstrate the advantages of using magnetic agarose resins in simple benchtop purifications as well as their research utility in the screening of recombinant antibodies using the Thermo Scientific Invitrogen™ ExpiCHO™ Expression Systems and the purification of recombinant proteins expressed by *in vitro* translation (cell-free) systems using the Thermo Scientific KingFisher™ Flex magnetic particle processor. Both the Protein AG and anti-DYKDDDDK magnetic agarose supports enabled isolation of >0.5mg protein per sample at >90% purity.

INTRODUCTION

Mammalian expression systems have become increasingly important to research scientists and biopharma production as they ensure a greater probability that the overexpressed protein is properly folded and contains relevant post-translational modifications such as disulfide bonds, phosphorylation events, and glycosylation structures. Whether mammalian cultured cells or *in vitro* translation (IVT) systems are used to overexpress the protein(s) of interest, downstream purification of that protein is often required before assessing its activity, performing structural studies, modifying the protein, or analyzing protein interactions.

Automating small-scale affinity purifications is helpful in handling multiple patient samples or libraries of overexpressed clones, in screening expression conditions, and in optimizing purification strategies. Magnetic beads are typically used as the purification support in these systems, but their relatively low binding capacity makes them better suited for analytical rather than preparative separations. Magnetic agarose provides a high-capacity solution to this problem and serves as a good tool to transition to future scale-up protocols.

Table 1. Characteristics of Pierce™ magnetic agarose

Composition	Magnetic, highly crosslinked agarose supports
Magnetization	Ferrimagnetic with low remanence
Mean Diameter	25 µm; (range 10-40 µm)
Bead concentration	25% slurry in PBS, 0.01% Tween-20, 0.05% NaN_3
Binding capacity	
Protein AG	>40 mg rabbit IgG/mL settled beads
anti-DYKDDDDK	>3.8 mg DYKDDDDK-tGFP/mL settled beads

Here we demonstrate a workflow for the automated purification of recombinant antibodies expressed in mammalian cultured cells and secreted into the media. We also show a similar workflow for the purification of DYKDDDDK-tagged fusion proteins that were translated *in vitro*. Both expression systems yield significant quantities of recombinant protein that showcase the utility of magnetic agarose as the appropriate purification support to efficiently capture and yield high microgram to low milligram protein quantities per sample.

MATERIALS AND METHODS

Protein Expression
Antibodies were expressed in ExpiCHO™ and Expi293™ Expression Systems per manufacturer's recommended guidelines. Recombinant DYKDDDDK-tagged fusion proteins were expressed with the Thermo Scientific 1-Step Human High-Yield Maxi *In Vitro* Translation Kit.

Antibody Purification
Following cell harvest, media supernatants from the ExpiCHO and Expi293 expressions were clarified by centrifugation and filtration through a 0.22µm filter per kit instructions. Aliquots (0.5mL and 1.0mL) of the clarified supernatants were purified on Pierce™ Protein AG Magnetic Agarose Beads manually or automated on either the KingFisher™ Duo or Flex Purification Systems. Eluted samples were neutralized and aliquots analyzed by SDS-PAGE and protein yields determined by A_{280nm} or by Detergent-Compatible Bradford Assay.

DYKDDDDK-tagged Protein Purification
Samples of the DYKDDDDK-tagged proteins in the IVT lysates were immunoprecipitated with Pierce™ Anti-DYKDDDDK Magnetic Agarose Beads or with Sigma Anti-FLAG™ M2 Magnetic Beads using the KingFisher™ Flex Purification System. Tagged proteins were eluted from the beads using a competing peptide of the tag sequence in triplicate

Western Blot Analysis
Eluted protein was analyzed by western blot using a Pierce™ Power Blotter, α-DYKDDDDK primary antibody, Invitrogen Goat Anti-Rabbit IgG Secondary Antibody, HRP, and Pierce™ SuperSignal West Dura Extended Duration Substrate.

Gel Analysis
Eluted protein was analyzed by either non-denaturing or denaturing gel electrophoresis and stained with coomassie blue or silver stain to monitor purification efficiency and purity.

RESULTS

Protein AG is a chimeric protein consisting of the five binding domains of Protein A and the two binding domains of Protein G to provide greater isotype selectivity on one purification support (Figure 1). This versatility enables purification of antibodies from the most common species with a single purification support (Figure 2, 3, and 4) in both manual and automated formats.

Figure 1. Protein A and G binding sites on antibodies

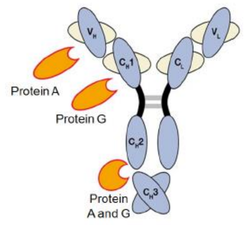
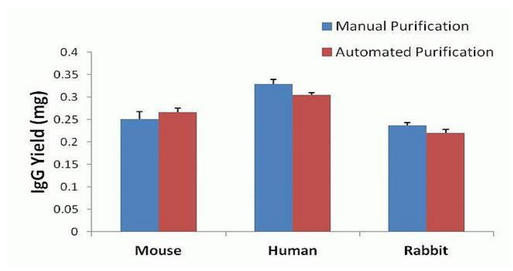


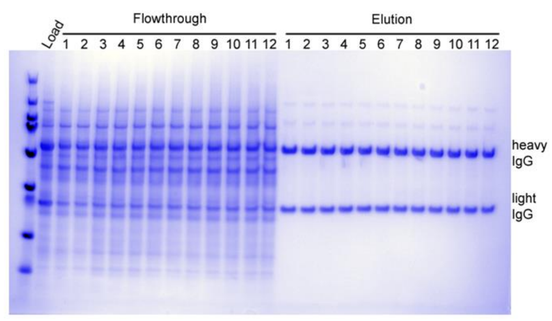
Figure 2. Similar Ab purification efficiency is achieved with manual and automated Protein AG purification protocols



Serum (50 µl) was diluted 10-fold with binding buffer and added to washed Protein AG magnetic agarose beads (10 µl). Antibodies were purified following the manual (using a magnetic stand) and automated (using KingFisher Flex) protocols provided. Antibody yield was estimated by absorbance at 280nm.

Figure 3. Automated purification of recombinant antibody from ExpiCHO media is robust and reproducible

A.

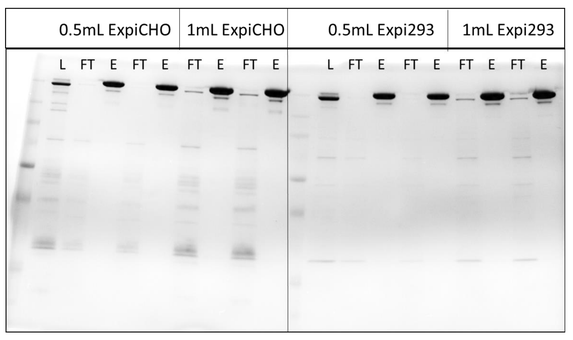


B.

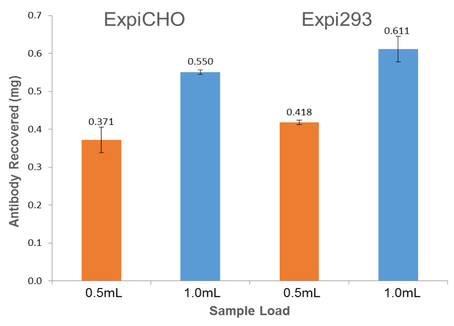
ExpiCHO media expressing humanized IgG (0.5 mL) was purified with Pierce Protein AG Magnetic Agarose beads (0.1 mL slurry) using an automated KingFisher Duo protocol. Load, flow-through and elution fractions were evaluated by reducing SDS-PAGE (panel A) to assess purity and binding efficiency. Total yield was estimated using the Pierce Detergent Compatible Bradford Assay and bovine gamma globulin as a standard (panel B). Average yield was 632 ± 55 µg per 0.5mL sample (8.8% CV).

Figure 4. Efficient Ab purification from both ExpiCHO and Expi293 high expression systems yields high-purity protein

A.



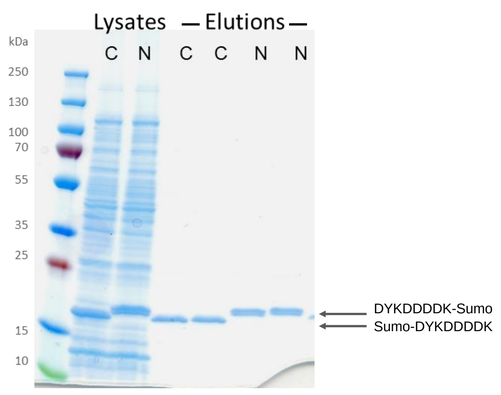
B.



Aliquots of ExpiCHO and Expi293 clarified media containing overexpressed anti-CD20 antibody (0.5 and 1.0 mL) were added to a 96-deep well plate, mixed with Protein AG magnetic agarose beads (40 µl of 25% suspension), washed, and eluted with 0.1M glycine, pH 2, using the KingFisher Duo Purification System. Panel A shows non-denaturing SDS-PAGE analysis of the starting supernatant load (L), flow-through (FT) or unbound fractions, and elutions (E) from the ExpiCHO and Expi293 samples. The high capacity Protein AG magnetic agarose purifications yielded 0.6 mg Ab per mL of media (Panel C) with CVs of <10%.

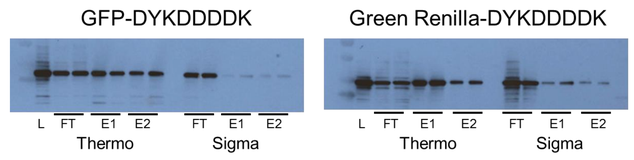
The epitope tag DYKDDDDK is commonly used in mammalian expression systems as a short affinity purification handle because it does not significantly change the characteristics or negatively impact the activity of the expressed protein. Since the tag is highly immunogenic, the corresponding antibodies raised against it provide a generic means of purification and detection of the tagged protein. The anti-DYKDDDDK magnetic agarose is a high capacity support that facilitates the purification of recombinant proteins in bacterial (Figure 5) and high-yield HeLA IVT lysates (Figure 6). The automated purification workflow incorporating competitive peptide elution preserved the activity of the DYKDDDDK-tagged proteins (Figure 7).

Figure 5. Anti-DYKDDDDK magnetic agarose efficiently captures both N-term and C-term tagged proteins



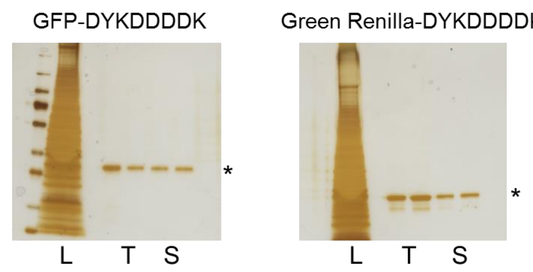
N-terminal and C-terminal DYKDDDDK-tagged Sumo protein overexpressed in *E. coli* was immunoprecipitated with anti-DYKDDDDK magnetic agarose (50 µl of a 25% suspension) using a manual protocol. Beads were washed and recombinant protein eluted with 0.1M glycine, pH 2.8. There is negligible non-specific binding to the magnetic agarose beads resulting in high purity protein recoveries.

Figure 6. Efficient purification of DYKDDDDK-tagged proteins expressed in HeLa IVT lysate



C-terminal DYKDDDDK-tagged tGFP and Green Renilla luciferase proteins were expressed in HeLA IVT lysate system and immunoprecipitated using Pierce™ Anti-DYKDDDDK Magnetic Agarose or Sigma Anti-FLAG™ M2 Magnetic Beads using the KingFisher™ Flex Purification System. Tagged proteins were competitively eluted with DYKDDDDK peptide and analyzed by Western Blot. Comparison of the starting lysate (L) and duplicate elution fractions show effective capture and elution of DYKDDDDK-tagged proteins.

Figure 7. Anti-DYKDDDDK magnetic agarose has low nonspecific protein binding



Purity of the DYKDDDDK-tagged tGFP and Green *Renilla* luciferase was confirmed by SDS-PAGE followed by silver stain. Starting load (L) and fractions eluted from the Thermo Anti-DyKDDDDK magnetic agarose (T) and the Sigma Anti-FLAG™ M2 magnetic beads (S) were run in parallel. Asterisks denote the location of the respective tagged proteins.

CONCLUSIONS

- Protein AG magnetic agarose is a versatile antibody purification support that can purify recombinant antibodies directly from clarified ExpiCHO and Expi293 culture media supernatants.
- The automated antibody purification protocol using the KingFisher Flex and Duo Systems efficiently captures and recovers all of the expressed antibody from the cell culture supernatants with minimal to no nonspecific background.
- Anti-DYKDDDDK magnetic agarose with competitive peptide elution can be used to purify DYKDDDDK-tagged recombinant proteins overexpressed in high-yield human IVT systems.
- Given the high expression yields (>500 µg per mL) of the HeLa IVT system, the anti-DYKDDDDK magnetic agarose is the best choice to provide enough capacity for automated, preparative purifications.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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