Automated Purification of Antibodies from CHO Media Using IMCStips® Containing Protein A Resin

IMCStips® Product:

Protein A

Description:

IMCStips® with the Hamilton® Microlab STAR workstation for the purification of proteins and immunoglobulins

Available Tip Size: 1 mL size

Catalog Numbers:

Resin Amount	Catalog Number Rack of 96
25 µL resin	04T-H6R80-1-25-96
50 µL resin	04T-H6R80-1-50-96

Method Benefits:

- High reproducibility
- Reduced hands on time
- Loose Dispersive Resin - maximum contact between resin and analyte
- Custom automated scripts available
- No additional equipment

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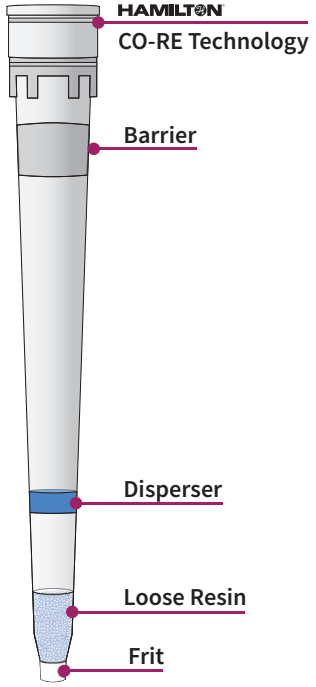
Introduction

Recombinant protein A (rPA) immobilized on chromatography media is used extensively for purification of immunoglobulins (IgGs) in many academic and industrial laboratories. Automating the purification process with rPA using a traditional column format or tip column format has been recently reported to allow for rapid interrogation of novel antibody candidates. In this prior study, the authors indicate that a packed bed tip column experiences some clogging issues with larger resin beds.

Here, we demonstrate the use of IMCStips to conduct INtip purification of IgGs using MabSelect SuRe LX resin on the Hamilton® Microlab STAR workstation. IMCStips are designed with Hamilton's CO-RE technology, providing an air-tight seal on the tips minimizing the mechanical stress during tip attachment and ejection. A large resin bed of 50 µL in 1 mL wide bore tip format is used to purify the recombinant IgG from CHO media, achieving ~80% recovery within 5 aspiration/dispense cycles and near complete recovery with increased aspiration and dispense cycles. Unlike conventional packed resins, the dispersive extraction technology of IMCStips leverage the turbulent mixing within the tips to increase interaction time and to improve extraction efficiencies. In comparison to the spin column formats, IMCStips in combination with the precise flow controls and repetitive aspirate and dispense cycles on the Hamilton Microlab STAR workstation provide higher yields and faster workflow speeds.



Deck Layout and Workflow for Protein A IMCStips®

Affinity Purification Method (Protein A)

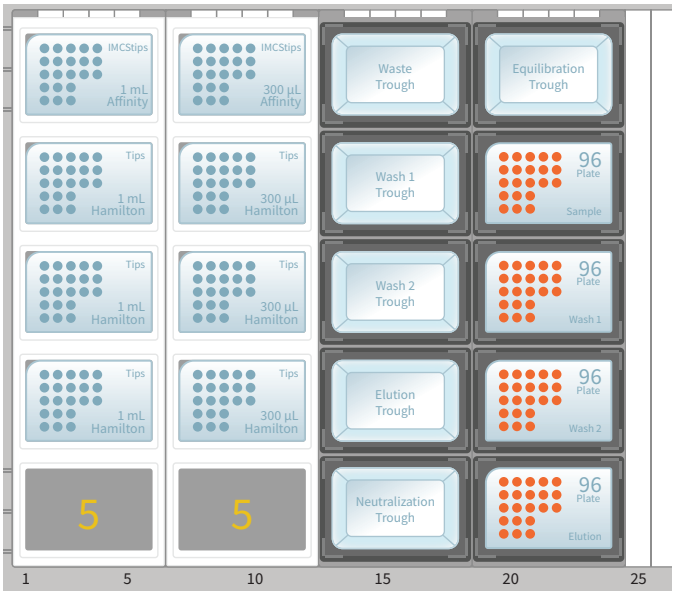


Figure 1. Typical deck layout for affinity purification followed by buffer exchange.

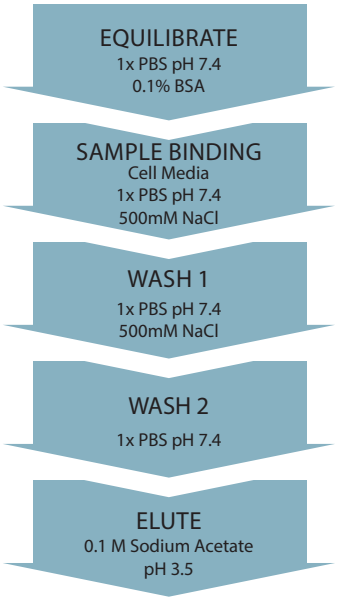


Figure 2. Workflow of Protein A based IgG purification using Hamilton Microlab STAR workstation.

Results

The development of the purification process was carried out using MabSelect SuRe LX resin (GE Healthcare) to purify humanized IgG1 expressed in CHO cells. The initial study compared the spin column-based purification method to the IMCStips INtip chemistry. For both approaches, the same sample volume of 1 mL was loaded onto the spin columns and IMCStips. Both spin columns and IMCStips were loaded with 50 µL of affinity resin. Spin column resin/samples were mixed by inversion prior to centrifugation. The flow through liquid was re-applied to the top of the spin column, inverted, then centrifuged. This process was repeated to mimic the repeated aspiration and dispense cycles performed during the INtip purification process. Re-applying the sample 5 times on the spin column did improve overall yield by 15-20% compared to 3 times. However, collecting the flow-through and re-applying the sample solution to the spin column requires more hands-on time, thus leading to longer sample preparation time. Despite the specific attempts to re-apply the samples multiple times on the spin column, the recovery was consistently higher for the INtip Affinity purification method by 20-30% (Figure 3).

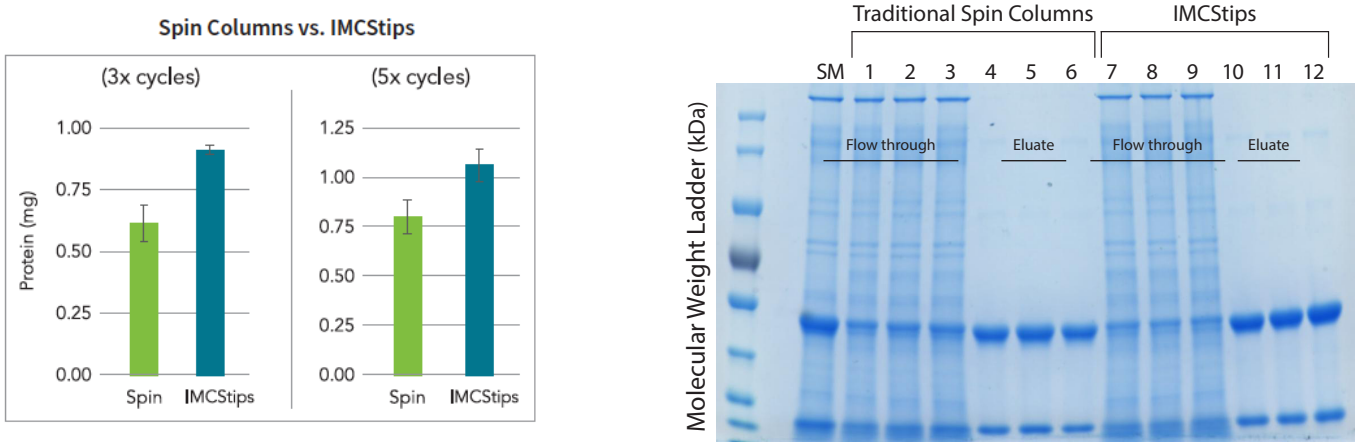
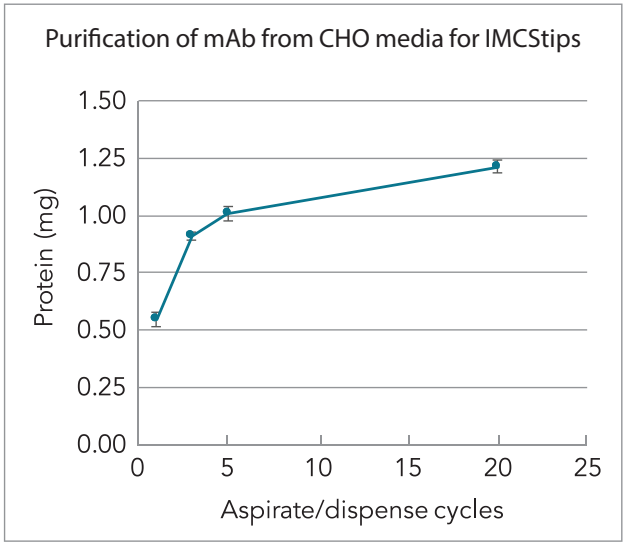


Figure 3. Purification of a single lot of CHO cell fermentation using MabSelect SuRe LX resin (50 µL resin bed) by centrifugation (spin) and IMCStips®. For consistency, the spin method utilized 200 x g RCF for 5 minutes and flow through samples were re-applied to the column either 3x or 5x, similar to the tip workflow. Spin format consistency yielded less mAb than tip based format, as indicated by both UV-Vis measurements (Abs @ 280 nm) and by SDS-PAGE.

Increasing the number of aspiration and dispense cycles was also tested for INtip Affinity purification, ranging from a single cycle up to 20 cycles. Approximately 70% of the target IgG was purified within 3 cycles, and 80% of IgG was purified within 5 cycles. While repeated pipetting cycles increased overall yield up to 1.25 mg, the time efficiency per microgram of purified protein is reduced after 5 cycles. (Figure 4).



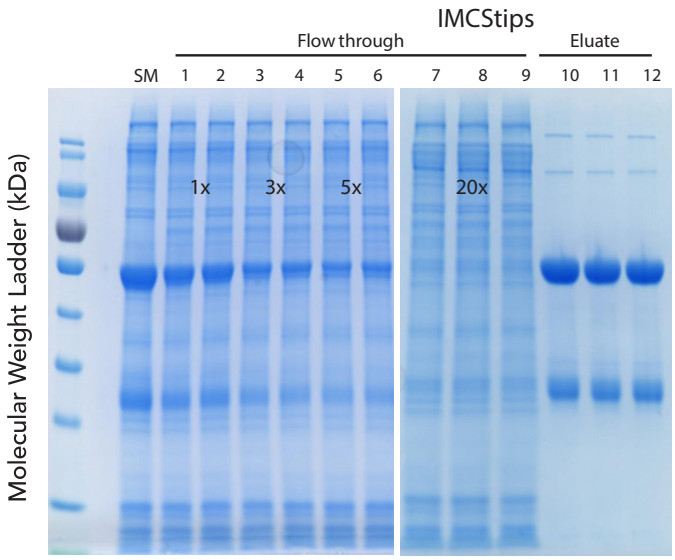
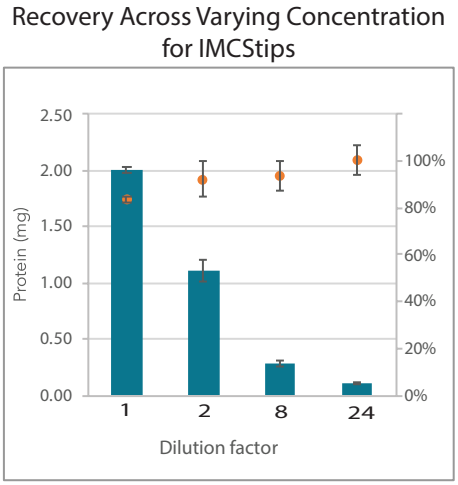


Figure 4. Increasing aspirate/dispense cycles improve the final yield of IgG as indicated by protein quantification by UV-Vis (Abs @ 280 nm) and also indicated by the depletion of the heavy and light chains in the flow through media. The eluants are after 20x aspirate/dispense cycles followed by a final elution volume of 400 µL in NaOAc pH 3.5. The sample was neutralized with 1/10 volume of 1 M Tris pH 8. Each cycle is a duplicate run with the exception of the final 20x flow through and the eluants in triplicates.

One of the advantages of dispersive extraction with IMCStips is the resin bed volume can be larger than traditional packed bed columns. The resin is not compacted and does not require higher pressures to achieve proper flow rates. Despite a larger resin bed of 50 uL, more than 90% recoveries were achieved across the different titers ranging from 2 mg/mL down to 0.083 mg/mL, which is over 20x fold dynamic range (Figure 5). The interday reproducibility for affinity purification for the same lot of cell media indicates less than 10% variance (Figure 6).



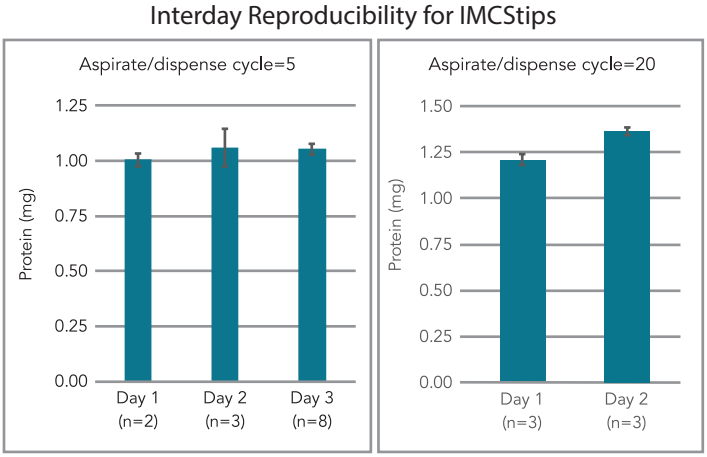


Figure 5. Serial diltutions of CHO media was prepared using 0.1% BSA in 1x PBS, pH 7.4 to assess capacities and recoveries across different concentrations.

Figure 6. The enrichment of mAb from cell media were tested over 3 days using different number of tips and two different aspirate/dispense cycles.

The quality of the antibody purified by IMCStips is consistent with conventional spin column format and FPLC workflow as shown on SEC and RP-UHPLC (Figure 7). All purification processes show low levels of aggregates and additional studies will be performed to further expand on this automated workflow.

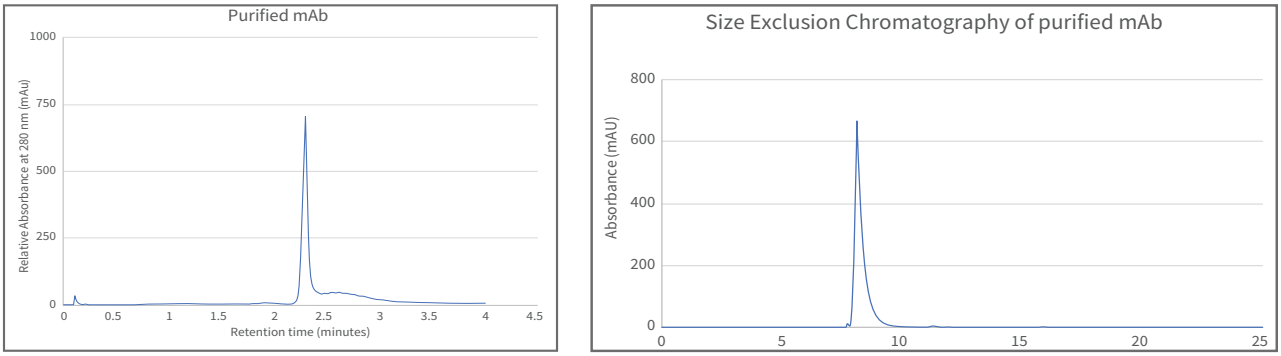


Figure 7. Analysis of the purified mAb using IMCStips® with rPA resin by reverse phase UHPLC (left) and by non-denaturing SEC (right) indicates similar profiles as conventional purification methods (not shown).

Materials and methods

Human antibody from serum and humanized antibody from CHO media
Human serum samples were obtained from healthy volunteers. Additional pooled human serum samples were purchased from Thermo Fisher. Humanized antibody expressed in CHO cells was provided by a collaborator. The media was centrifuged and filtered using a 0.45 micron PES membrane filter prior to loading onto IMCStips or on spin columns.

Conventional spin column purification of humanized IgG
An empty spin column fitted with 20 micron porosity was loaded with 50 µL of MabSelect SuRe LX resin. Based on the vendor specifications and in-house measurements, the slurry density was calculated to ensure that each spin column was loaded with 50 µL of resin. The resin was then washed with 500 µL of 1x PBS, pH 7.4 by pipetting the liquid on top of the resin. The samples were all centrifuged at 100 x g RCF for 2 minutes at room temperature. 1 mL of cell supernatant was loaded on each column and the flow through was re-applied either 3x or 5x to mimic the 3 to 5 aspiration/dispense cycles of a tip extraction process. The first wash solution was using 1x PBS, 500 mM NaCl, pH 7.4 followed by a second wash using 1x PBS, pH 7.4. The elution was using a single elution with 400 µL of 100 mM NaOAc buffer, pH 3.5. The eluate was immediately neutralized with 1/10th volume (40 µL) of 1 M Tris pH 8.

Graphical user interface to control aspirate and dispense cycles

Dispersive pipette extraction using IMCStips for purifying IgG on Hamilton Microlab STAR Workstation
IMCStips containing 50 µL resin bed of MabSelect SuRe LX are manufactured in-house and stored in 20% ethanol until use. The IMCStips containing the storage solution were loaded on the Hamilton deck. The 96 CO-RE head was used to dispense the storage solution into the waste trough. The IMCStips were then equilibrated with 1x PBS, pH 7.4 followed by sample binding, either a single aspirate/dispense cycle or multiple aspirate/dispense cycles (3, 5, 20). The wash, elution and neutralization solutions were identical to the spin column format as described above. The entire program and operation was conducted on a standard Hamilton Microlab STAR workstation with 4 channels and 1 CO-RE 96 head with standard labwares. No additional customized equipment or customized labware was used.

Automation:

HAMILTON

Hamilton® Microlab STAR Workstation: Automated Liquid Handler with a 96-Channel Multi-Probe head

Analysis of purified IgG by SEC and RP-UHPLC
Analytical SEC and RP was carried out on Vanquish UHPLC system with diode array detector. Approximately 2-5 microgram of purified IgG was loaded onto a MAbPac™ SEC-1 (Thermo Fisher) at 0.2 mL/min using 50 mM sodium phosphate, 300 mM NaCl as a running buffer. For RP analysis, the purified IgG was loaded on AdvanceBio RP-mAb C4, 2.1 x 50 mm, 3.5 µm (Agilent) using water, 0.1% TFA as mobile phase A and a blend of isopropanol, acetonitrile, water (70:20:10), 0.09% TFA as mobile phase B. The flow rate was 1 mL/min and gradient started at 15% B ramping up to 25% at 0.5 minute, 35% B at 1.5 minute, and 60% B at 3 minutes. The column was washed at 60% B for 1 minute and re-equilibrated to 15% for 1 minute.

Conclusion

Affinity enrichment of various target analytes is a routine process that is implemented throughout the early to late research phases. The work shown here is based on automation and high throughput purification using IMCStips with a large resin bed to achieve up to 2 mg of IgG purification in a single tip. The performance of the IMCStip is 20-30% superior to the spin column format and workflow speeds are faster than centrifugation methods. The dispersive design of IMCStips mixes the resin and sample, allowing large resin beds to be used in a narrow tip format and not sacrificing yields. Recoveries for concentrations ranging from 2 mg/mL down to 0.083 mg/mL were consistently above 90% as long as a passivating agent was used for low titer samples. The day-to-day reproducibility was demonstrated at less than 10%, and the entire workflow can be completed within 30 minutes or less if aspirate/dispense cycles are kept at less than 20 cycles. Overall, the performance of IMCStips with rPA immobilized resin (MabSelect SuRe LX) demonstrates top performance for purifying IgGs using automation for a fast, hands-free workflow.

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