Increased Throughput of INtip Affinity Purification for Larger Sample Volume

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INTRODUCTION

Dispersive pipette extraction is a patented INtip™ micro-extraction platform that leverages turbulent mixing of the resin within the pipette tip to increase interaction time between resin and sample. Coupling this core technology to the highly flexible, automated liquid handling system – Hamilton® Microlab STAR™ workstation – enables rapid INtip purification of antibodies, recombinant proteins and various other recombinant proteins in a consistent manner. Here, we address one of the major challenges in high throughput monoclonal antibody purification, which has been processing sample volumes over 3 mL using INtip micro-purification technologies. The workflow is outlined in ***Figure 1.***

Figure 1. *INtip chemistry workflow using Protein A affinity resin for purifying antibodies from cell supernatant.*

MATERIALS & METHODS

The automated workflows are generic templates for several different INtip chemistries to purify different types of targets, ranging from tagged recombinant proteins to immunoglobulins. These workflows can be combined with subsequent buffer exchange workflow using SizeX IMCStips. In this poster, we compared three different Protein A-based affinity resins to purify antibodies from 5 mL cell culture media to identify a resin with the highest mass transfer rate ***(Table 1)***.

Table 1*. Characteristics of three Protein A-based resins compared for INtip purification, according to manufacturers.*

	Resin A	Resin B	Resin C
Bead Chemistry	Cross-linked agarose	Cross-linked agarose	Methacrylic polymer
Bead Diameter (µm)	85	50	50
Protein A ligand	Tetramer of Z domain (modified B domain of Protein A)	Recombinant Protein A	Recombinant Protein A
Working pH range	3-12	3-10	1-13
Dynamic binding capacity	55 mg/mL for mAb	40 mg/mL for human IgG	54 mg/mL for polyclonal IgG

Figure 2*. Graphical user interface to control aspirate and dispense cycles. Optional steps can be selected to provide easy and flexible control of the affinity purification workflow.*

RESULTS

First, the method to determine binding cycle number and elution volume was optimized on 1 mL of antibody at known concentrations spiked in 1X PBS with 1 mg/mL BSA. Each IMCStips contains 50 mg of each resin, which has a theoretical binding capacity of 2 mg. At maximum antibody load, the optimal binding cycles are 30, which equals to approximately 30-minute resident time ***(Figure 3)***. However, at 15 binding cycles, all three resins recover > 70% of antibody. Resin C shows the highest human IgG recovery at all binding cycle numbers tested. Elution volumes were tested and 600 µL is the minimal volume that yields the most consistent recovery from IMCStips with 50 mg resin.

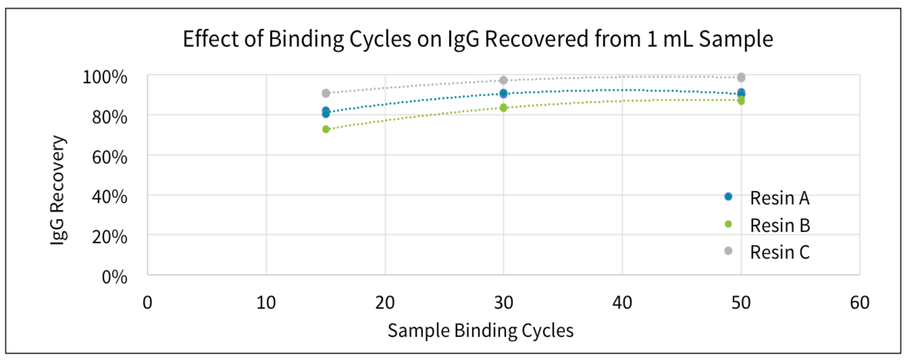

Figure 3*. Effect of IMCStips binding cycles on IgG recovery using three different Protein A resins. Starting material was 1 mL containing 2 mg of human IgG.*

Next, the purification method was performed on 1 mL spiked IgG at concentrations ranging from 0.1 mg/mL to 2 mg/mL which is the maximum binding capacities of these tips. Resin C recover > 80% of IgG in all starting concentrations tested ***(Figure 4)***.

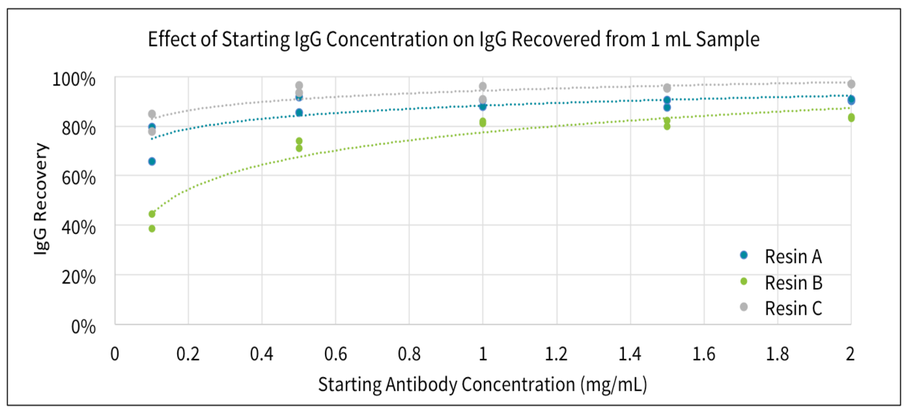

Figure 4*. Effect of starting IgG concentration in 1 mL sample on IgG recovery using three different Protein A resins. Starting material was 1 mL with concentrations ranging from 0.1 to 2 mg/mL of human IgG.*

For purification of antibody from > 3 mL media, we tested two automated methods. One is bulk binding, which uses 1 mL IMCStips to bind directly in 5 mL starting material. Another method is batch binding, which transfers each mL to a separate 96-well plate to bind at a time.

IgG recovery was calculated using a known concentration of IgG spiked into 1X PBS with 1 mg/mL BSA. In this case, the IgG concentration was at 0.18 mg/mL to match CHO culture media supernatant used in later experiment.

The bulk binding method shows significant IgG recovery difference between the three resins tested with Resin C having the highest recovery at 80% ***(Figure 5A)***. This involves binding cycles of 50 in 5 mL of starting solution, so IgG is depleted during the binding event, leading to diminishing binding efficiency towards the end of binding process. On the other hand, batch binding resulted in > 80% recovery from all three resins ***(Figure 5B)***. Resin C recovered significantly more than Resin B using batch binding method. When compared between two different binding methods, batch binding shows significantly higher recovery when Resin A and Resin B were used, while there is a minor improvement with Resin C ***(Figure 5C)***.

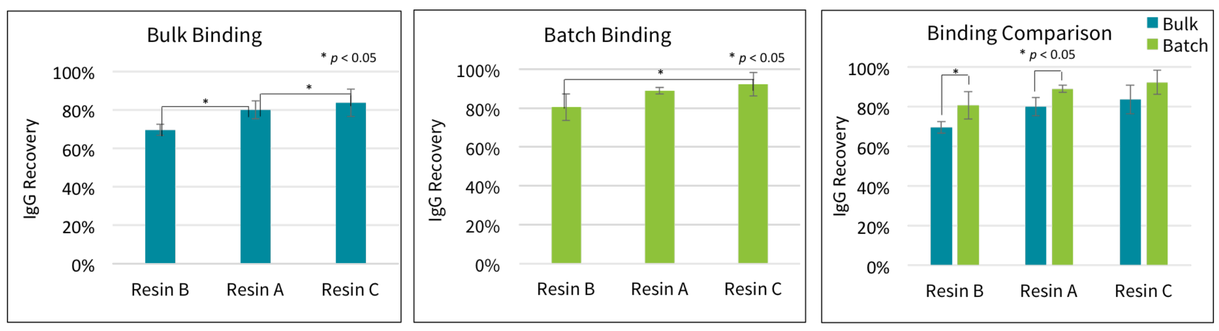

Figure 5*. Effect of bulk or batch binding methods on IgG recovery using three different Protein A resins. Starting material was 5 mL of 0.18 mg/mL human IgG spiked in 1X PBS with 1 mg/mL BSA.*

INtip purification was repeated on 5 mL CHO culture media with an estimated titer of 0.18 mg/mL recombinant human IgG1. This time, IMCStips were conditioned in 1X PBS, instead of 1 mg/mL BSA in 1X PBS. Again, bulk and batch binding methods were tested. In bulk binding, Resin A and Resin C had > 60% recovery which was significantly higher than Resin B at 45% ***(Figure 6A)***. Batch binding showed that Resin C recovered higher IgG than the other two resins ***(Figure 6B)***. When comparing between the two binding methods, each resin demonstrated higher recovery on different binding methods and there was no clear trend to which binding method performs better ***(Figure 6C)***.

Lower recovery from culture media compared to spiked sample could be due to IMCStips conditioning step without 1 mg/mL BSA. The depletion of antibody in culture media after INtip purification was confirmed on SDS-PAGE shown in ***Figure 7***. Purified antibody from bulk binding method shows > 99% purity.

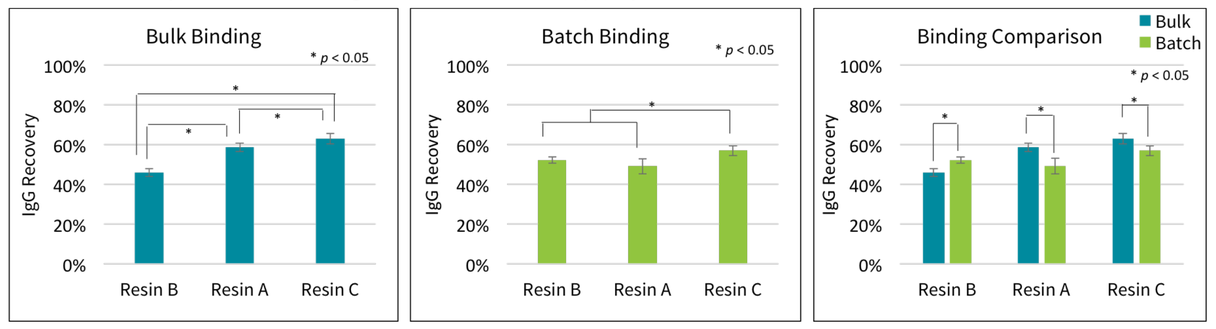

Figure 6*. Effect of bulk and batch binding methods on IgG recovery using three different Protein A resins. Starting material was 5 mL of CHO culture media with an estimated titer of 0.18 mg/mL recombinant human IgG1.*

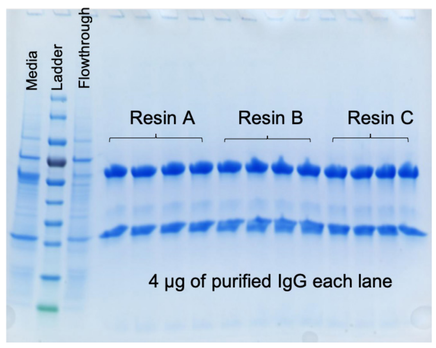

Figure 7*. SDS-PAGE of purified recombinant human IgG1 from 5 mL of CHO culture media using bulk binding method. Purified antibody was loaded at 4 µg per lane. The same volume of starting media and flowthrough were loaded to show antibody depletion.*

CONCLUSION

We compared three different Protein A-based affinity resins to purify antibodies from 5 mL cell culture media to identify a resin with highest mass transfer rate. Resin C has shown higher mass transfer than other two resins. It has the highest recovery even at 0.1 mg/mL IgG starting concentration. Using this resin, the INtip purification can be speed up by 20-30% via reduction in binding cycles or resident time. We have also shown here that INtip antibody purification can be applied to sample volume > 3 mL with recovered IgG amount over 400 µg and > 99% purity. The recovery can be further improved by minimizing non-specific binding, such as pre-conditioning IMCStips with 1 mg/mL BSA.

The automated INtip purification program shown here processes 5 mL cell media in 24-well plate using 1 mL IMCStips on 96-MPH. The process takes 1 hour going from 24 different cell media to 24 purified proteins. The method can be scaled up to 192 x 5 mL samples on a single Microlab STAR workstation with minimal human intervention for total processing time of less than 8 hours. Proposed deck layout is illustrated in ***Figure 8***.

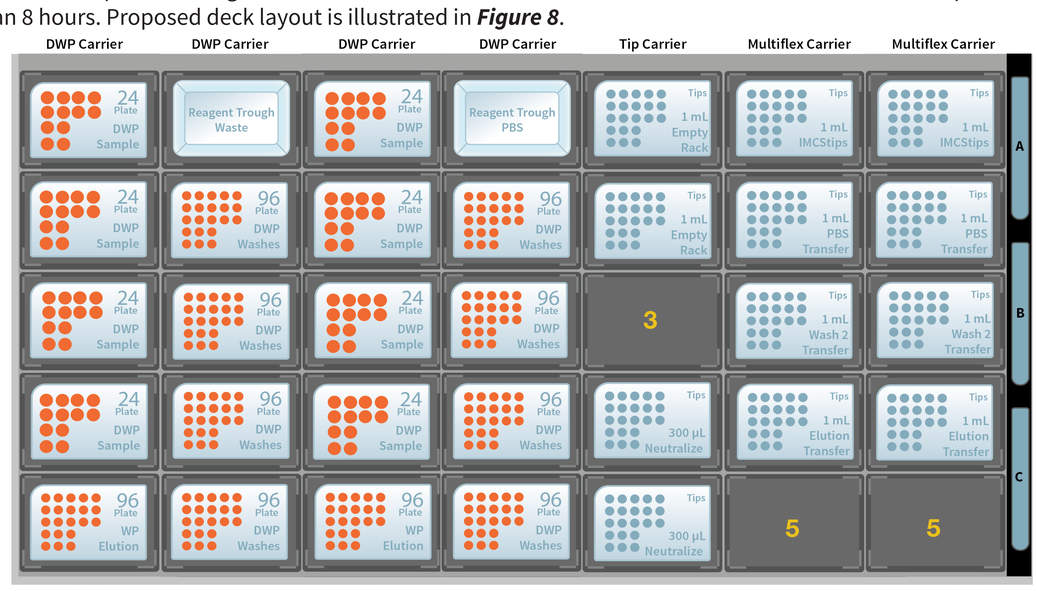

Figure 8*. Proposed deck layout on a single Hamilton STAR workstation to automate antibody purification from 192 x 5 mL samples using IMCStips in less than 8 hours.*

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