# Affinity Purification Followed by Buffer Exchange Using INtip dSPE on an Automated Liquid Handler for High Throughput Protein Purifications

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## INTRODUCTION

Protein purification lies as a bottleneck in biotechnology and biotherapeutic characterizations. Pipette based dispersive solid phase extraction (dSPE), or INtip dSPE leverages automated liquid handlers for dispersive micro-extractions for samples such as recombinant proteins, antibodies, and oligonucleotides in high-throughput applications. Incorporation of INtip dSPE reduces the need for ancillary equipment, such as centrifuge, vacuum manifolds, or magnetic plates with grippers to support the sample extraction process. Here, we demonstrate two different chromatography techniques (affinity and size exclusion) on multiple protein targets on the automated liquid handler. The automated workflow starts with affinity extraction of proteins from crude lysates, followed by rapid buffer exchange on a size exclusion pipette. The workflow is outlined in ***Figure 1.***

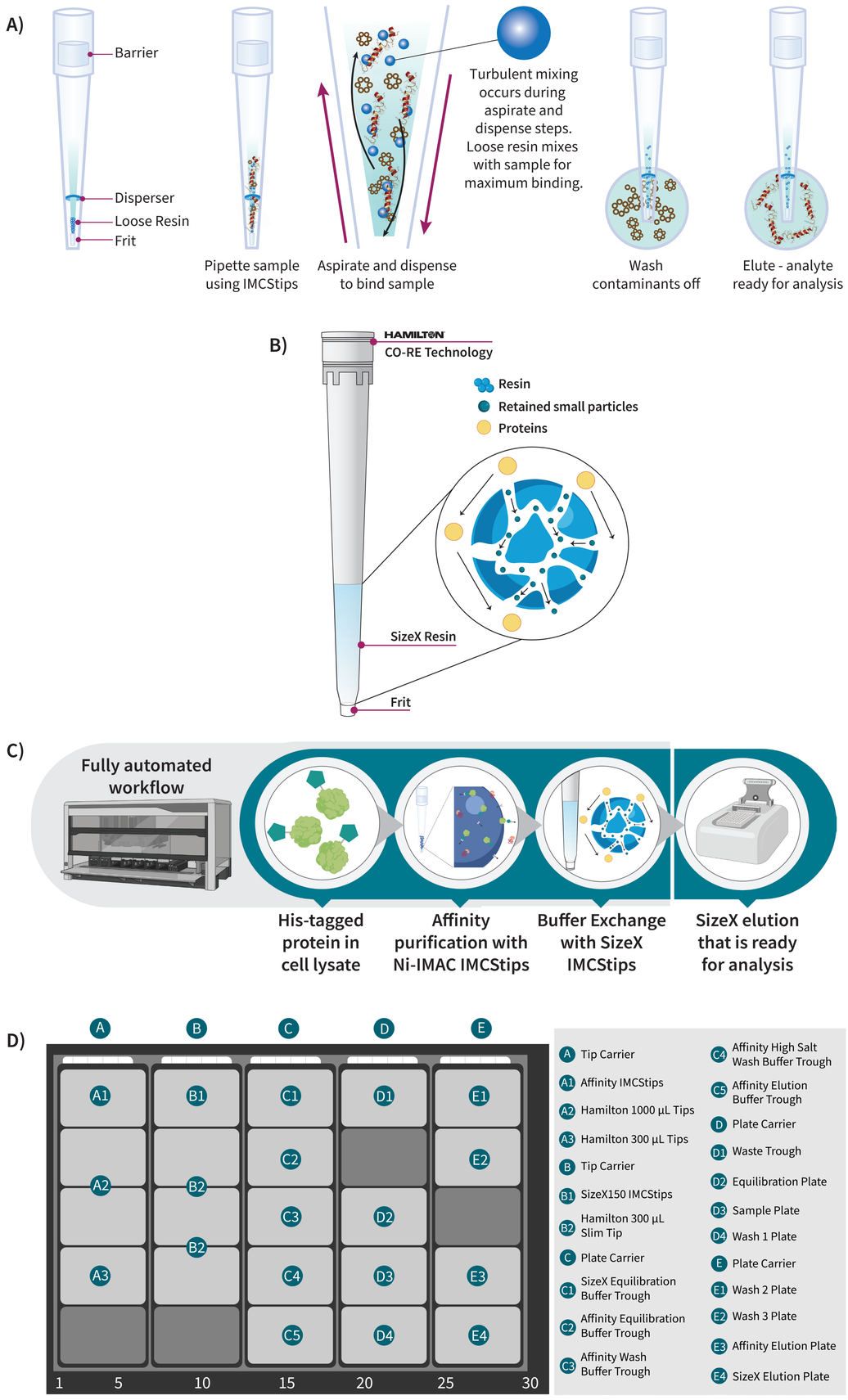


***Figure 1.*** *(A) IMCStips® containing loose resin employ INtip dSPE to perform efficient automated extractions. Ni-IMAC resin was used in the IMCStip® to purify his-tagged proteins from cell lysate.* ***(B)*** *SizeX IMCStips® contain inert size exclusion resin and are used to buffer exchange protein samples.* ***(C)*** *The sample preparation workflow includes affinity purification using Ni-IMAC IMCStips® followed by buffer exchange using SizeX$_{150}$ IMCStips® which is fully automated on a Hamilton STAR system. The resulting purified proteins are ready for downstream analysis in less than 90 minutes.* ***(D)*** *Layout of the hardware and consumables to run affinity and buffer exchange in single run.*

## MATERIALS AND METHODS

IMCStips® are available with different affinity resins (Ni-IMAC for his-tagged proteins, protein A/G hybrids for immunoglobulins, streptavidin for biotinylated targets) to target the protein of interest. SizeX IMCStips® (IMCS, Irmo, SC) are micro-SEC in pipette tips, compatible on Hamilton liquid handlers.

IMAC-Sepharose 6 Fast Flow (6FF) resin (Cytiva, Uppsala, Sweden) was packed in 1 mL wide bore tips (Hamilton) by IMCS and charged with Ni (P/N 04T-H6-R72-1-15 -8 or -96). Three polyhistidine-tagged proteins, Dasher GFP (GFP), beta-glucuronidase (BGUS), and arylsulfatase (PAS) with different molecular weights (27, 71 and 61 kDa) were recombinant proteins in 1x PBS.

SizeX$_{150}$ IMCStips® (IMCS, P/N 04T-H6-R76-0A-350-8 or -96) were used. All three proteins were diluted and used at 4 mg/mL. Extinction coefficients (ε280) for the three proteins, GFP, BGUS, and PAS are 25.12, 140.57, and 102.79 $M^{-1}cm^{-1}$, respectively. Buffer exchange results were from four biological replicates. Protein concentrations in eluates were measured by A280 on a NanoDrop 2000. Recovered volumes were measured using a handheld pipette.

Desalting efficiency was determined using imidazole in 1x PBS. Standard curve of imidazole was established across a concentration range from 1 to 200 mM using A230 on NanoDrop. 300 mM imidazole in 50 mM HEPES, pH 8 was loaded on SizeX$_{150}$ IMCStips®, and the eluate was measured on NanoDrop. For desalting efficiency, 300 mM of imidazole was loaded on the column, and its detection in the protein fraction based on its absorbance at 230 nm was below the limit of quantitation (< 1 mM), which is > 99.7% effective for desalting such molecules. Ethanol and cobalt chloride retention were also measured to similar levels (data not shown). Enzyme activity assays were done with p-catechol sulfate for PaS.[1]

## RESULTS

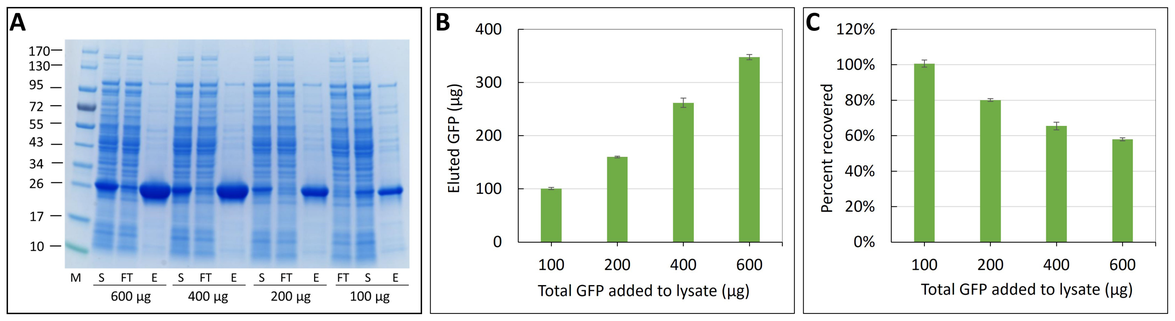


***Figure 2.*** *The affinity purification of GFP using 15 µL Ni6FF resin bed in 1 mL IMCStips® at four different protein quantities in bacterial lysate are shown.* ***(A)*** *SDS-PAGE of the starting material (S), the flow-through (FT) which is the sample post processing with affinity tips, and eluate (E) across the four different starting amounts suggest proportional increase in yields. Note that FT and S positions are not in same sequence along the gel as the other three sample sets.* ***(B)*** *Quantified proteins in the elution fraction increase with sample loads.* ***(C)*** *Percent recoveries decrease with increasing starting amounts, with near complete capture and recovery for the lowest protein amount and less than 60% recovery for the highest protein load.*

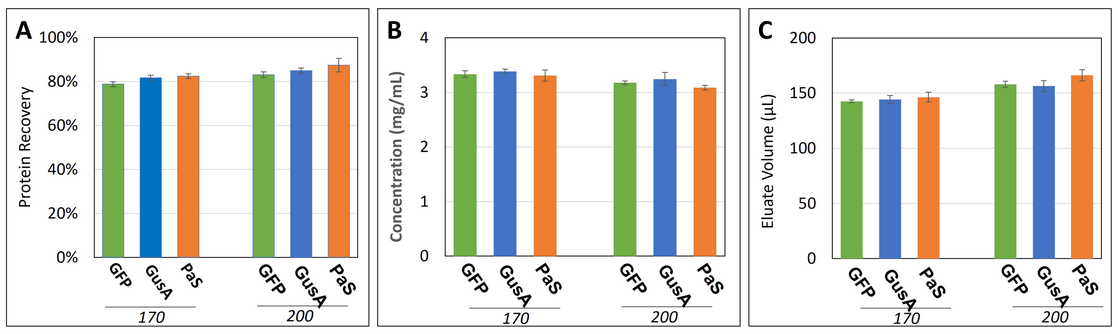


***Figure 3.*** *The recoveries of three different proteins on SizeX$_{150}$ using two different chaser dispense volumes, which refers to the plunger displacement volume to push air through the sample and resin.* ***(A)*** *The protein recoveries hover around low 80% when using 170 µL as the chaser dispense volume, and slight increase for all three proteins are observed when using 200 µL chaser dispense volume.* ***(B)*** *The starting concentrations for all three proteins are 4 mg/mL, and the recovered concentrations are lower, which is expected when running size exclusion chromatography for desalting. The higher concentration is obtained when using smaller chaser dispense volume.* ***(C)*** *The eluate volumes correlate with the chaser dispense volumes, with less than 150 µL recovered when using smaller chaser dispense volume and slightly over 150 µL with chaser dispense volume of 200 µL.*

**Table 1.** SizeX$_{150}$ buffer exchange settings using GFP

| Parameters | Values | | |
|---|---|---|---|
| Sample volume (µL) | | 150 | |
| Sample load volume (µL) | | 80 | |
| Chaser volume (µL) | | 150 | |
| Chaser dispense volume (µL) | 150 | 170 | 200 |
| Recovery (%) ± stdev (n = 4) | 76 ± 2% | 78 ± 3% | 83 ± 2% |
| Elution volumes (µL) | 132 ± 3 | 141 ± 7 | 156 ± 1 |
| Protein conc. (mg/mL) | 3.43 ± 0.01 | 3.31 ± 0.11 | 3.15 ± 0.05 |

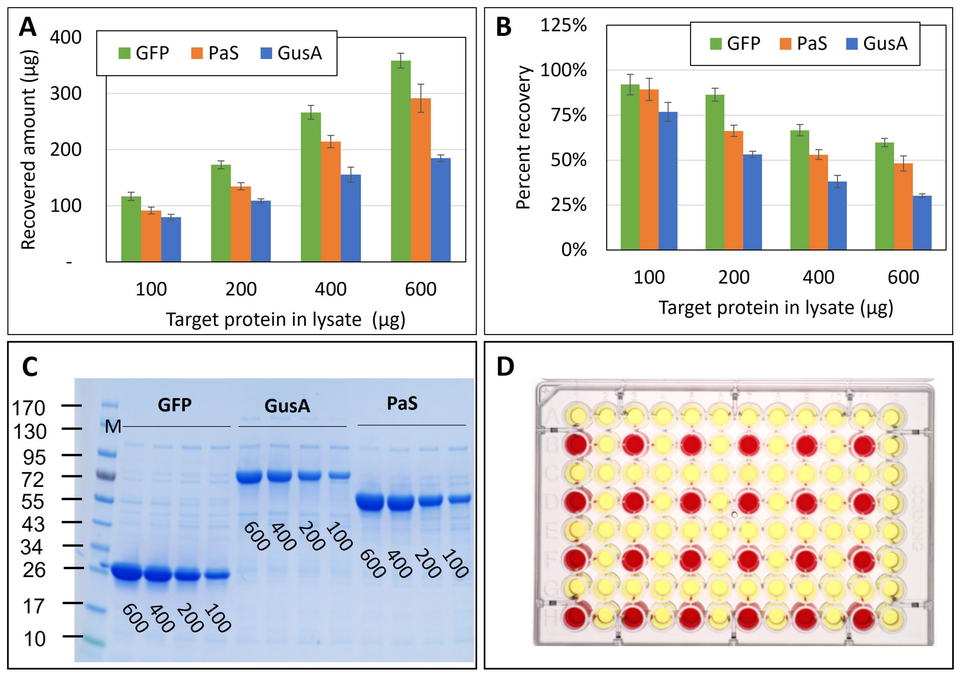


***Figure 4.*** *The combined chromatography methods of affinity purification and buffer exchange on automated liquid handler can be used for high throughput workflows.* ***(A)*** *Histidine labeled proteins (GFP, PaS, GusA) were added into bacterial lysates and purified with Ni-IMAC in pipette format.* ***(B)*** *The recoveries for 15 µL resin bed was more optimal for 100 and 200 µg, whereas more resin will assist with higher recoveries for larger quantities (400 and 600 µg).* ***(C)*** *SDS PAGE of the proteins at different quantities match the Bradford quantifications.* ***(D)*** *Purified PaS are active after undergoing the two step purifications on the liquid handler, and there is no detectable cross over between wells or tips.*

## CONCLUSION

- Two different chromatography techniques are run in sequence on 96 samples
- Affinity purification followed by buffer exchange yields purified proteins in target buffer for easier downstream analytics
- 96 samples purified and desalted under 90 minutes on a single automated system
- Similar approaches are routinely used for antibody purification at milligram quantities for later stage discovery

## REFERENCES

1. Dubois, G.; Turpin, J.; Baumann, N. P-Nitrocatechol sulfate for arylsulfatase assay: detection of metachromatic leukodystrophy variants. *Adv Exp Med Biol* **1976,** *68*, 233-7.

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