Affinity-based Dispersive Pipette Extraction for Automated Purification

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ABSTRACT

Affinity-based extractions in complex matrixes are typically time-consuming due to the labor intensive work of hands on pipetting and centrifugation. A typical spin column affinity extraction takes an hour to bind the sample and requires excess buffer solutions to wash off non-specific binding proteins using multiple centrifugation steps. A quick, high throughput affinity-based enrichment method for target proteins is desired to allow for quicker screening of samples. We report a high-throughput sample preparation method using antibody and his-tag based enrichment on a robotic liquid handling system with dispersive pipette extraction. Specifically, Protein A and cobalt-IMAC resins were used for IgG enrichment from human serum and his-tagged proteins from cell lysates, respectively. The Protein A extractions were compared side-by-side with a traditional spin-column based extraction protocol. Two different his-tagged proteins were purified from cell lysate using the IMAC resins in tips. These developments allow for completely automated 30 minute sample preparation leading to high-throughput sample screening and better compatibility with in-house automated liquid handling systems.

INTRODUCTION

Affinity-based extractions in complex matrixes are typically time-consuming due to the labor intensive work of hands on pipetting and centrifugation. A typical spin column affinity extraction takes an hour to bind the sample and requires excess buffer solutions to wash off non-specific binding proteins using multiple centrifugation steps. A quick, high throughput affinity-based enrichment method for target proteins is desired to allow for quicker screening of samples. Herein, we report a high-throughput sample preparation method using antibody and his-tag based enrichment on a robotic liquid handling system with dispersive pipette extraction.

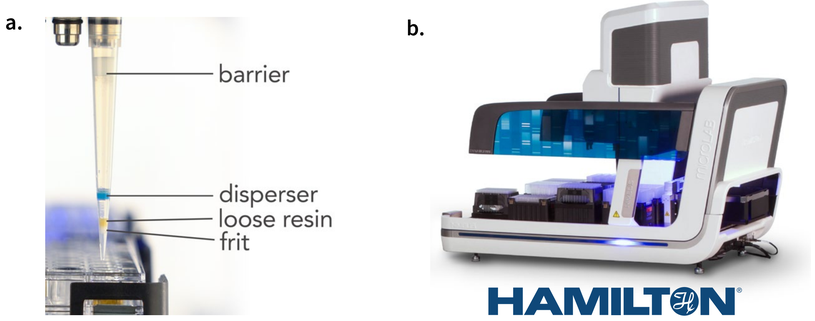


Figure 1. (a) *Components in dispersive pipette extraction* (b) *Microlab® NIMBUS® from Hamilton Robotics*

Figure 2. Affinity IMCStips with cobalt-IMAC resin on VIAFLO 96 from Integra

MATERIALS AND METHOD

IMCS Protein A tips on automatic pipetting system

- 10 µL of serum
- 20 µL of 50% MabSelect SuRe™ LX slurry
- Binding buffer: 20 mM sodium phosphate, 0.15 M sodium chloride
- Elution buffer: 0.1 M sodium citrate, pH 3.5
- Neutralization buffer: 1 M tris, pH 9.0

IMCS cobalt-IMAC tips on automatic pipetting system

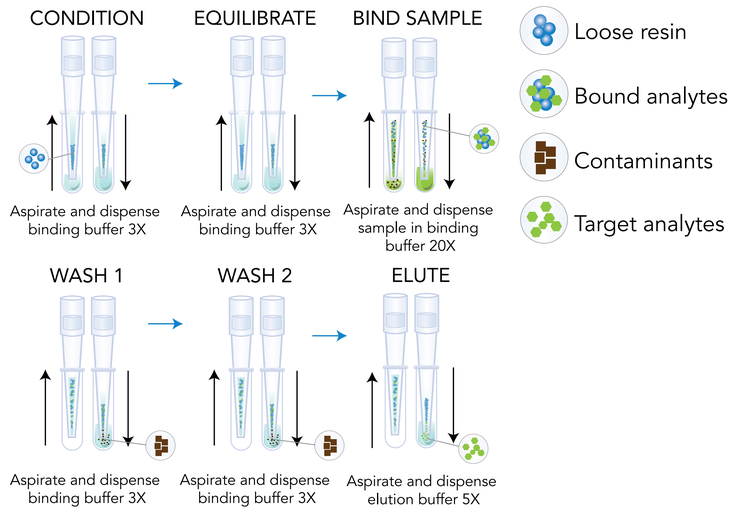
- 1 mg of cell lysate
- 50 µL of 50% cobalt-IMAC slurry
- Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 20 to 40 mM imidazole, pH 7.4
- Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

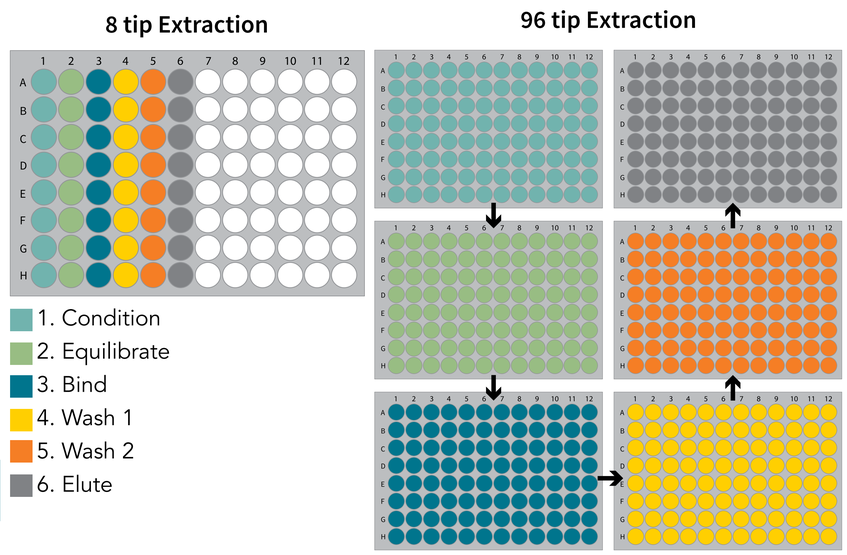
Gel Imaging and Western Blotting of purified protein samples

- Bio-Rad Mini-Protean Tetra System
- FisherBiotech FB300 Power Supply
- Immobilon-FL Transfer Membrane
- Mini-Protean TGX Precast Gels
- iBind Western Device and Cards
- Thermo Scientific™ Pierce™ Purified Recombinant Protein A and Conjugates
- Pierce ECL Western Blotting Substrate
- Amersham Imager 600

Workflow for 300 µL IMCStips

1. Condition: Aspirate 250 µL of Binding Buffer - 3x
2. Equilibrate: Aspirate 250 µL of Binding Buffer - 3x
3. Binding Sample: Aspirate 200 µL of Sample in Binding Buffer - 20x
4. Wash 1: Aspirate 250 µL of Binding Buffer - 3x
5. Wash 2: Aspirate 250 µL of Binding Buffer - 3x
6. Elution: Aspirate 100 µL of Elution Buffer - 5x





RESULTS

Immunoglobulins from human serum samples were purified using IMCStips loaded with resin modified with Protein A on multi-channel liquid handling system (Nimbus and VIAFLO 96) within 30 minutes from start to finish. The extraction process in comparison to conventional spin column format, did not require additional incubation time and recoveries were comparable or better than conventional formats (***Figure 3***). His-tagged enzyme was also purified from crude cell lysates (***Figure 6***) in similar fashion on both multi-channel liquid handling systems within 30 minutes using IMAC resin. The final purity of the target enzymes (> 80%) are shown in Figure 4b. Further optimization of target proteins could be achieved by modifying the wash buffer compositions to achieve higher purity.

The workflow demonstrated here show the potential to achieve 96 sample purification within 30 minutes by leveraging dispersive pipette extraction on multi-channel liquid handling systems.

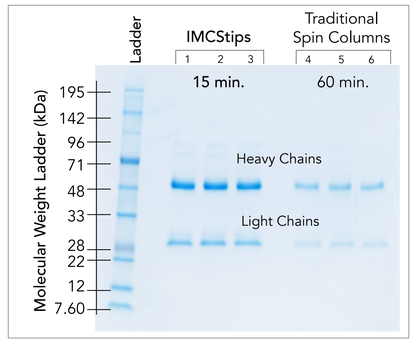


Figure 3. Side by side comparison of Protein A IMCStip elution and Protein A spin column. 20 µL of resin slurry was used for 10 µL of human serum.

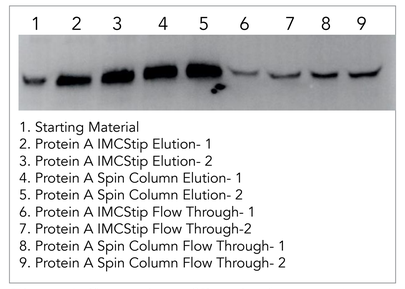


Figure 4. Comparison of Protein A extracted samples using Protein A IMCStip and Protein A spin column on a Western blot.

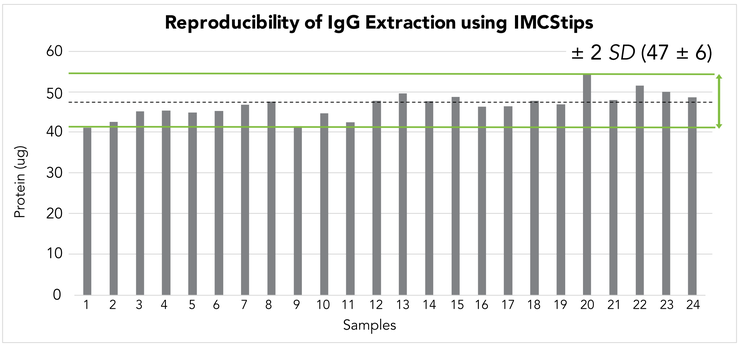


Figure 5. A comparison of protein recovery for 24 serum samples enriched using the 300 µL Protein A IMCStip. These samples were biological replicates to show the reproducibility of the extraction. The calculated CV value for this sample set was 6.5%

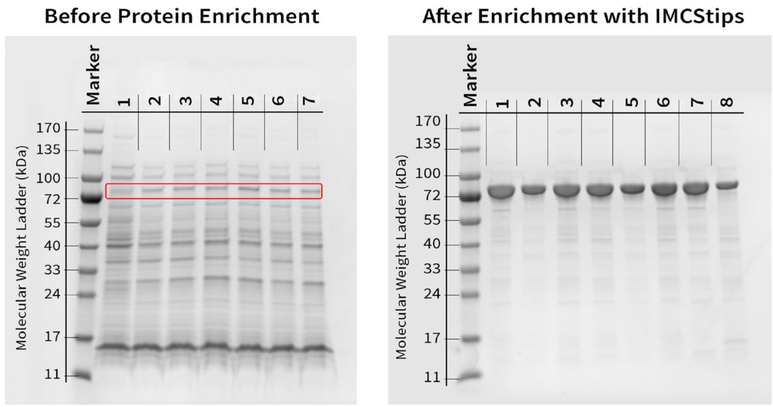


Figure 6. Before and after enrichment of 8 different recombinant his-tagged proteins in lysate using the cobalt-IMAC IMCStips.

CONCLUSIONS

We successfully developed a fully automated high-throughput affinity based protein extraction from complex matrices. These findings will allow for 30 minute complete sample preparation leading faster screening and downstream applications.

Next steps are to use different types of affinity resins in order to purify different targets in complex matrixes.

REFERENCES

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