APPLICATION NOTE

Affinity purification

IMCStips™ Product:

Description: IMCStips on Integra Viaflo96 for purification of his-tagged proteins and immunoglobulins

Cobalt-IMAC Tips
Resin Code: R71

300 µL or 1 mL

Protein A Tips
Resin Code: R80

300 µL , or 1 mL

Method Benefits

- High reproducibility
- Reduced hands on time
- Loose Dispersive Resin - maximum contact between resin and analyte
- Easily integrates with automated platform
- No additional equipment

Ordering Information

www.imcstips.com/imcstips
888-560-2073
inquiries@imcstips.com

Affinity-based dispersive pipette extraction of IgG or his-tagged proteins on Integra Viaflo96

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Introduction

Affinity extractions are typically time-consuming processes involving prolonged incubation steps and multiple wash steps. When launching the initial extraction protocols, the initial hands-on time with multiple pipetting and centrifugation steps can be tedious. A typical spin column affinity extraction takes an hour to bind the target analyte and requires excess buffer solutions to wash off non-specific binding proteins with multiple centrifugation steps. Magnetic bead-based assays still require longer incubation times and multiple liquid transfer steps as wash steps. Dispersive pipette extraction based affinity purification is demonstrated using two resin types on the Integra Viaflo96 system. The dispersive pipette extraction leverages turbulent mixing to increase contact time between resin and analyte, which reduces the overall incubation time required to achieve effective extractions.

Materials:

Protein A IMCStips

- 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

Cobalt-IMAC IMCStips

- 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

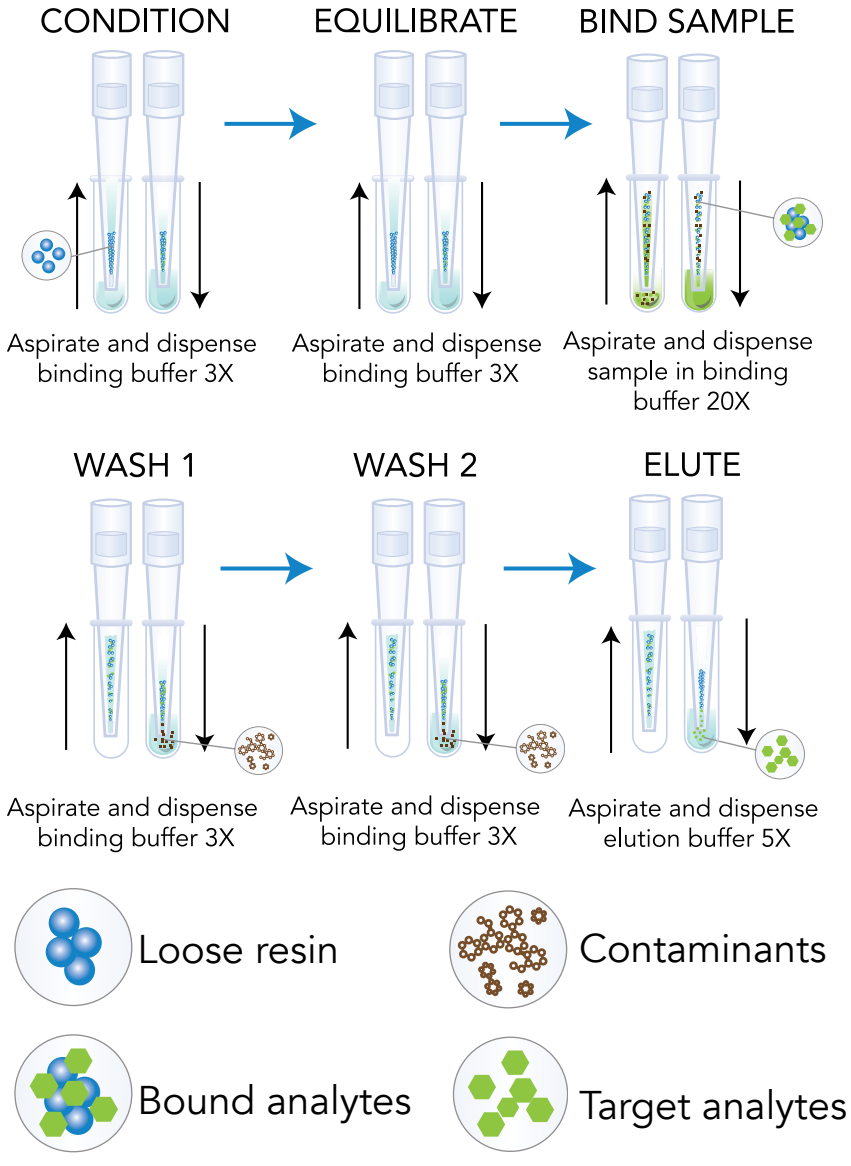
Semi-Automation:

INTEGRA

Integra Viaflo96

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



Method

Resin slurries were purchased from GE Life Sciences, MabSelect SuRe™ and HisPur cobalt IMAC. Serum samples were obtained from a healthy volunteer with written consent. For immunoglobulin purification and his-tagged protein purifications, the buffers were used according to the vendor recommendations. SDS-PAGE of serum samples, cell lysates and purified IgG or purified his-tagged proteins were performed on Bio-rad Mini- Protean Tetra system using TGX 4-15% gradient pre-cast gels. Each lane was loaded with approximately 5-10 microgram of protein. Gels were imaged on an Amersham Imager 600. Thermo Scientific Coomassie Blue was used for protein quantification. For Western blots, PDVF membranes were used and blots were blocked with heat denatured BSA solution using iBind Western Device (Life Technologies).

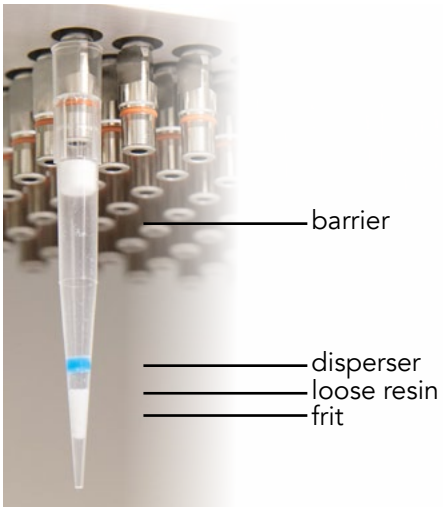


Figure 1. Components in dispersive pipette extraction

Results

Using Protein A IMCStips with a Integra Viaflo96 system, the target protein was enriched within 30 minutes. The extraction was compared with a conventional spin-column based extraction. Also, using the Cobalt-IMAC IMCStips, a his-tagged β-Glucuronidase was purified from cell lysate. The extracted purified proteins were shown using 1-D gel imaging and western-blotting.

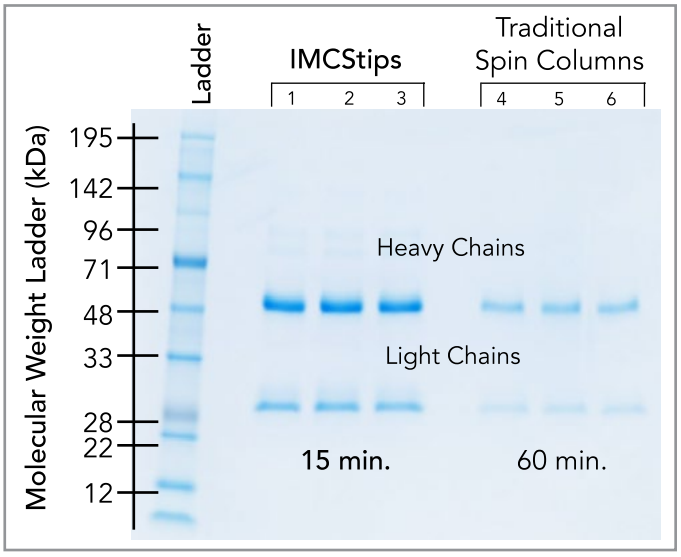


Figure 2. Side by side comparison of Protein A IMCStip elution and Protein A spin column. 20 µL of resin slurry was used for 10 µL of human serum.

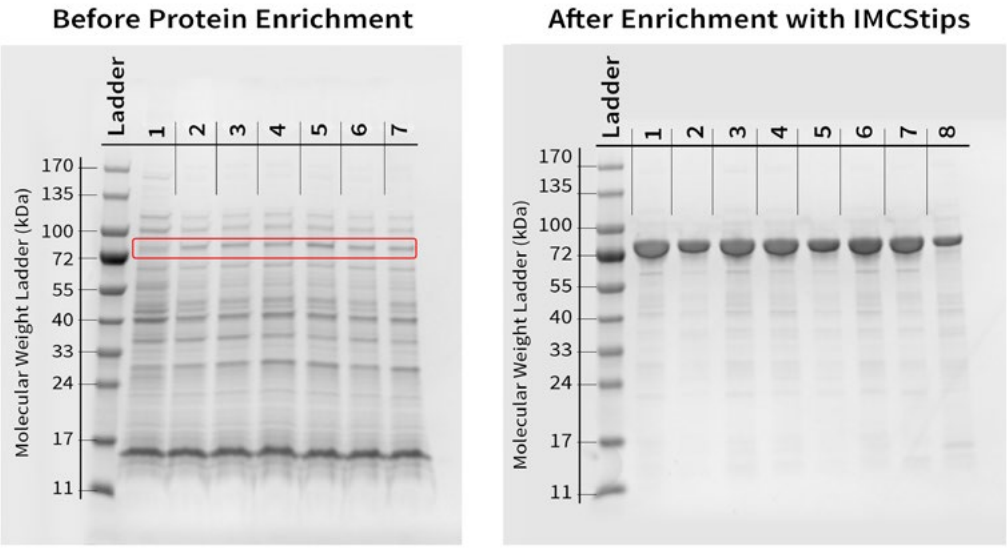


Figure 4. Before and after enrichment of 8 different recombinant his-tagged proteins in lysate using the Cobalt-IMAC IMCStips.

Conclusion

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

For R&D use only. Not for use in diagnostic procedures.

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