Automating Lower Throughput Repetitive and Tedious Protein Purifications with a Variable Span Pipetting System

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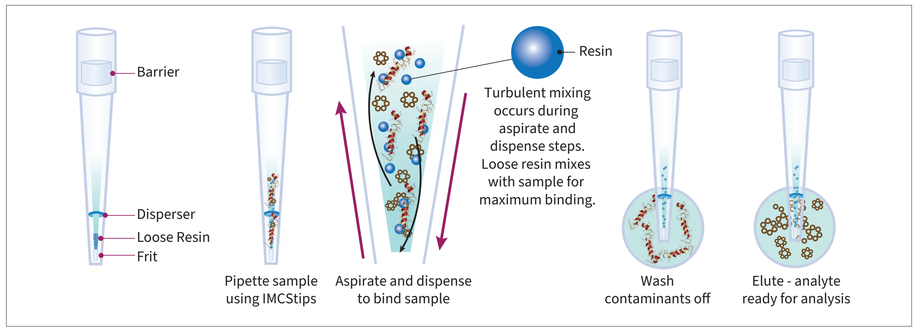
INTRODUCTION

- Semi-automated affinity purification on INTEGRA ASSIST+.
- Standardizing varied sample containers with lower throughput.
- Lower cost, semi-automated workflow for IgG purification.

Figure 1: *1 mL INTEGRA IMCStips® with protein A affinity resin uses patented dispersive solid-phase extraction (dSPE) technology. Turbulent mixing during repeated aspirate and dispense steps using the INTEGRA ASSIST+ pipetting robot results in optimal sample binding.*



METHODS

Instrumentation

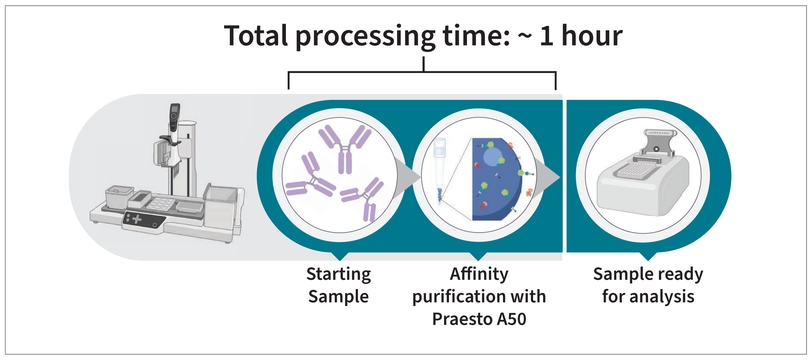


Figure 2: *Semi-automated workflow using 1 mL INTEGRA IMCStips with Praesto A50 resin for affinity purification. The samples were processed in less than one hour.*

INTEGRA ASSIST+ with a 6-channel Voyager was used to purify samples in microfuge tubes (**Figure 3A**). An INTEGRA ASSIST+ with the 8-channel Voyager was used to transfer these samples into a 96-well plate (**Figure 3B**). The INTEGRA VIAFLO96 was used to purify the 48 samples with IMCStips (**Figure 3C-D**). The entire workflow was programmed in VIALINK.

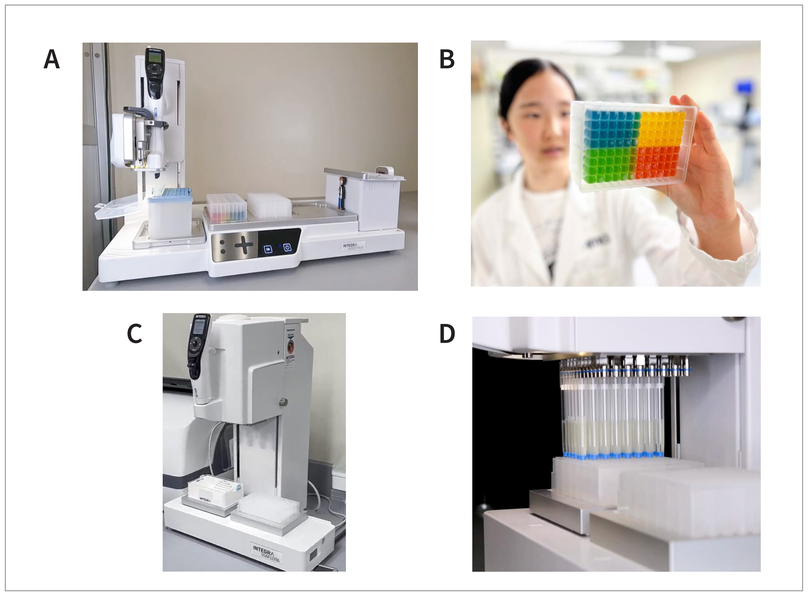


Figure 3. *Instrument setup for the experiment.*

Preparations

A single column of 12 filtered standard tips was arranged vertically (**A**). The reagent buffers were placed in position A of an 8-well trough (**B**). Next, five columns of microfuge tubes were placed on the deck (**C**). Finally, six IMCStips were placed in the second column of the tip box, beside the standard filtered tips (**D**). The storage solution was removed before running the affinity protocol.

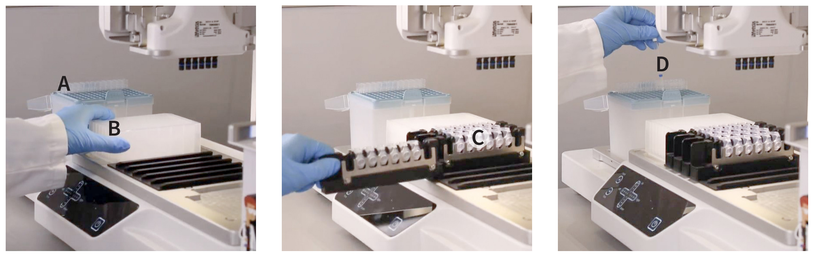


Figure 4. *Photographs of the consumables and materials required for the experiment.*

Recovery profile

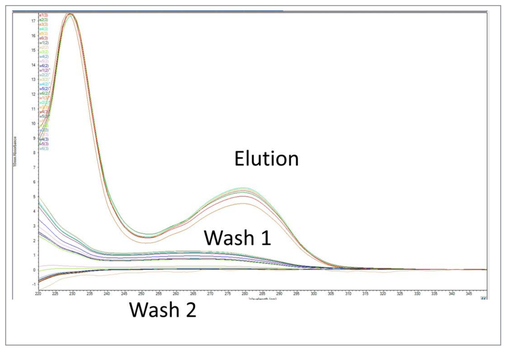
Initial method

- 1250 µL IMCStips with 25 µL Praesto A50.
- 900 µL cell culture supernatant.
- 30 cycles of binding (80 µL/s aspirate, 20 µL/s dispense).
- 2 washes (900 µL, 1x PBS, pH 7.4).
- Elution 150 µL, 100 mM glycine, pH 2.5.
- Neutralize w/ 5 µL, 1 M Tris-HCl, pH 8.8.

Sample ID	Protein Conc (mg/mL)
e1	5.3
e2	5.3
e3	5.5
e4	5.4
e5	5.2
e6	5.6

Average: 5.4 mg/mL
St Dev: ± 0.14 mg/mL
RSD: 3%

UV traces of washes and eluates



Sample ID	Wash 1 (Abs)	Wash 2 (Abs)
1	0.55	0.019
2	0.602	0.026
3	0.663	0.026
4	0.581	0.021
5	0.603	0.032
6	0.591	-0.027

RESULTS

Method programming

Equilibration

Program steps

No.	Action	Summary
1	Tip Load	
2	Aspirate	450µl / Track. 0.0mm
3	Move (X,Z)	X: 146.7mm Z: 27mm
4	Aspirate	800µl / Track. 12.0mm
5	Delay	9s
6	Dispense	800µl / Track. 12.0mm
7	Delay	10s
8	Delay	10s
9	Loop	Step: 4 Count: 3
10	Move Z	Z: 36mm
11	Dispense	150µl / Track. 0.0mm
12	Delay	10s
13	Move (X,Z)	X: 142.8mm Z: 38.9mm
14	Move Z	Z: 55mm

- Aspirated a large air volume (450 µL) to use for additional air blow out
- Incremental blow outs used (50-100 µL) after equilibration, sample binding, washes and elution
- Equilibration step (800 µL)
- Added blowout after equilibration step

- Lowered pipetting speeds to 20 µL/s aspirate, 15 µL/s dispense.

Sample binding

Program steps

No.	Action	Summary
15	Tip Spacing	13.5mm
16	Aspirate	150µl / Track. 0.0mm
17	Move (X,Z)	X: 232.6mm Z: 27mm
18	Aspirate	800µl / Track. 14.5mm
19	Delay	7s
20	Dispense	800µl / Track. 14.5mm
21	Delay	10s
22	Delay	10s
23	Loop	Step: 18 Count: 30
24	Move Z	Z: 40mm
25	Dispense	200µl / Track. 0.0mm
26	Delay	10s

- Adjust spacing to fit with microfuge tubes
- Pre-aspirate with additional dispense volume
- Sample binding (800 µL)
- Hold time increased to 20 s after dispense
- Additional 50 µL from prior 450 µL aspiration step

Wash 1

Program steps

No.	Action	Summary
27	Move (X,Z)	X: 237.2mm Z: 43.8mm
28	Move Z	Z: 55mm
29	Aspirate	150µl / Track. 0.0mm
30	Move (X,Z)	X: 254.8mm Z: 29mm
31	Aspirate	850µl / Track. 15.0mm
32	Delay	7s
33	Dispense	850µl / Track. 15.0mm
34	Delay	10s
35	Delay	10s
36	Loop	Step: 31 Count: 3
37	Move Z	Z: 43mm
38	Dispense	200µl / Track. 0.0mm
39	Delay	10s

- Wash (850 µL)
- Additional 50 µL from prior 450 µL aspiration step

Wash 2

Program steps

No.	Action	Summary
40	Move (X,Z)	X: 258mm Z: 41.4mm
41	Move Z	Z: 55mm
42	Aspirate	150µl / Track. 0.0mm
43	Move (X,Z)	X: 275.7mm Z: 30mm
44	Aspirate	850µl / Track. 15.0mm
45	Delay	5s
46	Dispense	850µl / Track. 15.0mm
47	Delay	10s
48	Delay	10s
49	Loop	Step: 44 Count: 2
50	Move Z	Z: 42mm
51	Dispense	250µl / Track. 0.0mm
52	Delay	10s

- Wash (850 µL)
- Additional 100 µL from prior 450 µL aspiration step

Elution

Program steps

No.	Action	Summary
53	Move (X,Z)	X: 280.1mm Z: 42.6mm
54	Move Z	Z: 55mm
55	Aspirate	100µl / Track. 0.0mm
56	Move (X,Z)	X: 297mm Z: 18mm
57	Aspirate	125µl / Track. 4.0mm
58	Delay	5s
59	Dispense	125µl / Track. 4.0mm
60	Delay	10s
61	Loop	Step: 57 Count: 5
62	Move Z	Z: 25mm
63	Dispense	200µl / Track. 0.0mm
64	Delay	10s
65	Move (X,Z)	X: 300.4mm Z: 21.9mm
66	Move (X,Z)	X: 293.6mm Z: 39.4mm
67	Move Z	Z: 100mm

- Mixing (125 µL) in 150 µL volume
- Additional 100 µL from prior 450 µL aspiration step

CONCLUSION

- Slower flow rates were needed to avoid liquid build up.
- Longer delays were included to ensure complete liquid movement.
- Recovery of 0.5 to 1.0 mg IgG using IMCStips filled with 25 µL of Praesto A50.
- Low background by UV-Vis on 2nd wash.
- RSD at 3% for eluates.

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