Comparative IMCStip-Based Protein A Purification on the Tecan Fluent: Impact of Resin and Elution pH on Antibody Aggregation

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INTRODUCTION

- Optimization of protein purification is critical for obtaining high recovery, purity, and monomer percentage. The optimization process is generally a time consuming and manual process. Several variables need to be accounted for including:
  - Resin type
  - Resin binding capacity
  - Wash/Elution conditions
- In this study, IMCStips are used for the purification of two antibody samples. For the optimization of protein purification, two commercially available resins were used:
  - MabSelect™ PrismA: Industry standard, high binding capacity, specificity
  - Praesto™ Jetted A50 HipH: Known for higher pH elution
- Use of Tecan IMCStips in a fully automated antibody purification and buffer exchange process on the Tecan Fluent platform
- Key findings:
  - pH effect: Similar recovery at pH 3.6; higher recoveries with Praesto™ Jetted A50 HipH at pH 3.9-4.5 vs MabSelect™ PrismA
  - Monomer percentage: Resin- and pH-specific effects

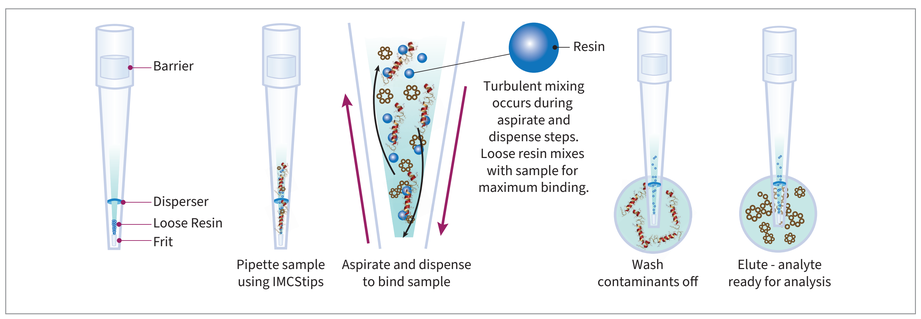

Figure 1: *IMCStips® containing loose resin employ dSPE to perform efficient automated extractions.*

METHODS

- Fully automated Protein A purification and buffer exchange performed on the Tecan Fluent system
  - 1 mL Protein A Tecan IMCStip
    - 25 µL MabSelect™ PrismA
    - 25 µL Praesto™ Jetted A50 HipH
  - 1 mL SizeX$_{150}$ Tecan IMCStip
- Monoclonal antibodies captured from clarified cell culture supernatants using MabSelect™ PrismA and Praesto™ Jetted A50 HipH
- Elution pH range: 2.9 to 4.9
- Analyzed purified antibody samples for recovery and monomer percentage using:
  - Absorbance at 280 nm
  - SDS-PAGE
  - Size-exclusion chromatography (SEC)

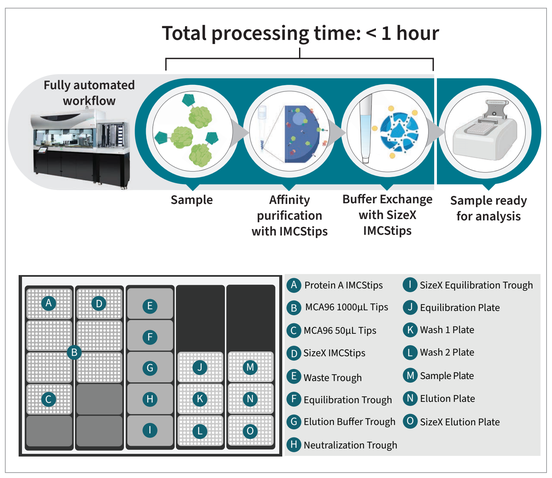

Figure 2: *Sample deck layout for automated protein purification followed by buffer exchange of 96 samples using IMCStips on the Tecan Fluent.*

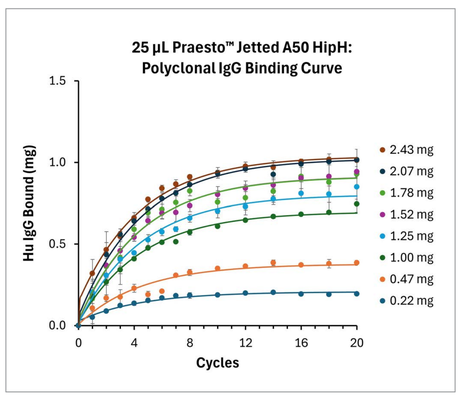

Figure 3: *Binding kinetics for 25 µL HipH 1 mL Tecan IMCStips.*

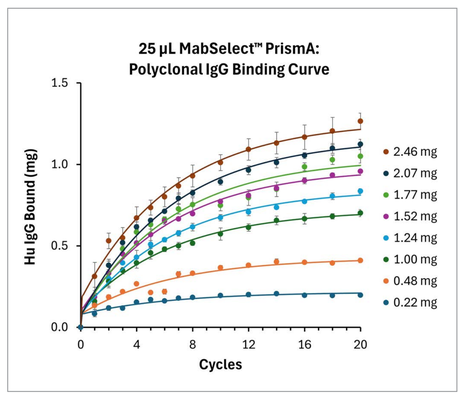

Figure 4: *Binding kinetics for 25 µL PrismA 1 mL Tecan IMCStips.*

MabSelect™ PrismA v Praesto™ Jetted A50 HipH: Recovery Comparison

MabSelect™ PrismA v Praesto™ Jetted A50 HipH: mAb			
Resin Type	Elution pH	Total Protein, µg	Monomer Percentage, %
MabSelect™ PrismA	2.9	181.2	97%
MabSelect™ PrismA	3.2	176.4	98%
MabSelect™ PrismA	3.4	176.3	98%
MabSelect™ PrismA	3.6	167.7	99%
MabSelect™ PrismA	3.9	53.3	100%
MabSelect™ PrismA	4.2	2.3	100%
MabSelect™ PrismA	4.4	4.6	N/A
MabSelect™ PrismA	4.7	0.0	N/A
MabSelect™ PrismA	4.9	0.0	N/A
Praesto™ Jetted A50 HipH	2.9	164.0	98%
Praesto™ Jetted A50 HipH	3.2	169.8	98%
Praesto™ Jetted A50 HipH	3.4	167.6	98%
Praesto™ Jetted A50 HipH	3.6	170.0	99%
Praesto™ Jetted A50 HipH	3.9	159.5	100%
Praesto™ Jetted A50 HipH	4.2	143.7	100%
Praesto™ Jetted A50 HipH	4.4	109.6	100%
Praesto™ Jetted A50 HipH	4.7	77.9	100%
Praesto™ Jetted A50 HipH	4.9	22.4	100%

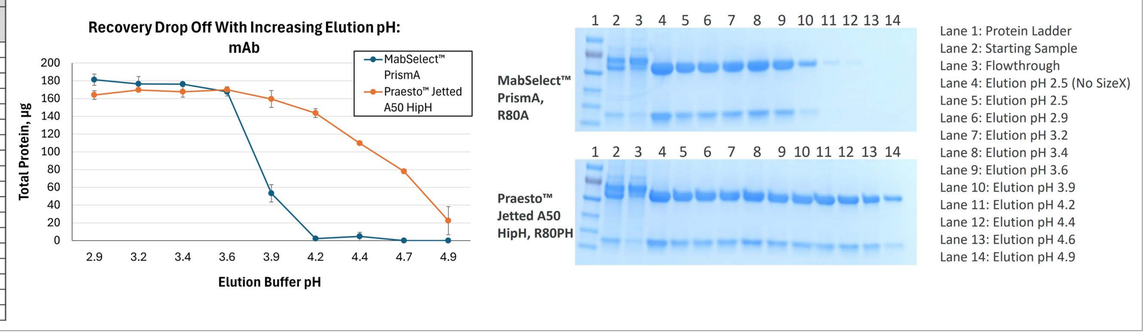

Figure 5: *Comparison of recovery between MabSelect™ PrismA and Praesto™ Jetted A50 HipH with different elution buffer pH's. For the purification, a monoclonal antibody expressed in cell culture supernatant was used.*

Recovery With Increasing Elution pH: Bispecific mAb			
Resin Type	Elution pH	Total Protein, µg	Monomer Percentage, %
MabSelect™ PrismA	2.9	828.0	90.2
MabSelect™ PrismA	3.2	846.9	89.8
MabSelect™ PrismA	3.4	764.7	90.3
MabSelect™ PrismA	3.6	809.9	90.3
MabSelect™ PrismA	3.9	760.7	92.5
MabSelect™ PrismA	4.2	503.9	95.8
MabSelect™ PrismA	4.4	343.2	97.9
MabSelect™ PrismA	4.7	172.8	98.8
MabSelect™ PrismA	4.9	92.6	98.9
Praesto™ Jetted A50 HipH	2.9	701.8	87.9
Praesto™ Jetted A50 HipH	3.2	673.5	87.6
Praesto™ Jetted A50 HipH	3.4	616.7	87.6
Praesto™ Jetted A50 HipH	3.6	624.2	88.5
Praesto™ Jetted A50 HipH	3.9	645.5	89.0
Praesto™ Jetted A50 HipH	4.2	589.0	89.9
Praesto™ Jetted A50 HipH	4.4	543.2	91.9
Praesto™ Jetted A50 HipH	4.7	537.7	92.8
Praesto™ Jetted A50 HipH	4.9	394.2	94.2

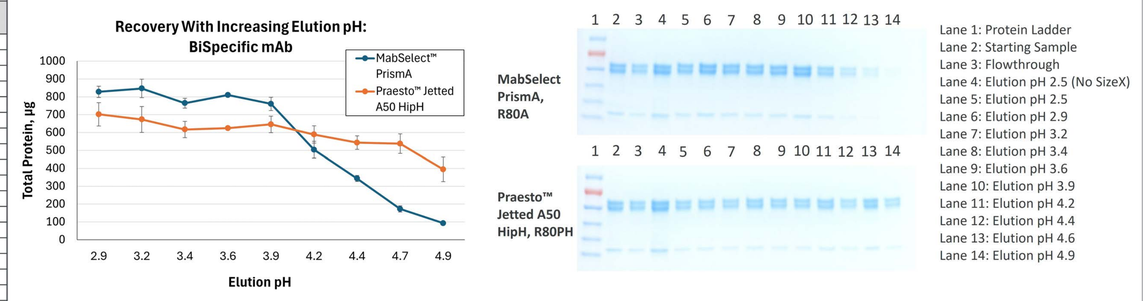

Figure 6: *Comparison of recovery between MabSelect™ PrismA and Praesto™ Jetted A50 HipH with different elution buffer pH's. For the purification, a bispecific monoclonal antibody expressed in cell culture supernatant was used.*

mAb: MabSelect™ PrismA

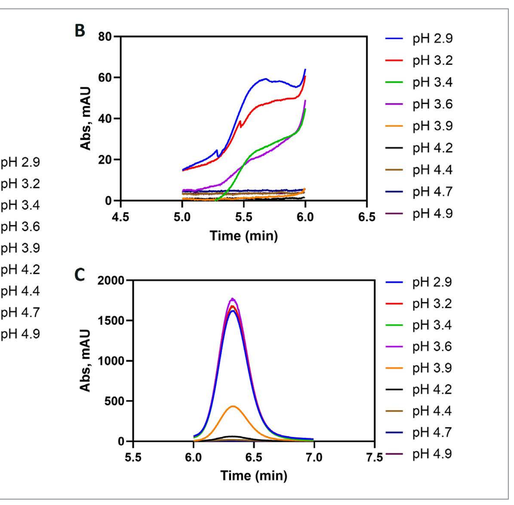

Figure 7: *Comparison of chromatography from MabSelect™ PrismA purified mAb eluted at different pHs. A) Full chromatograph, B) Aggregate, C) Monomer.*

mAb: Praesto™ Jetted A50 HipH

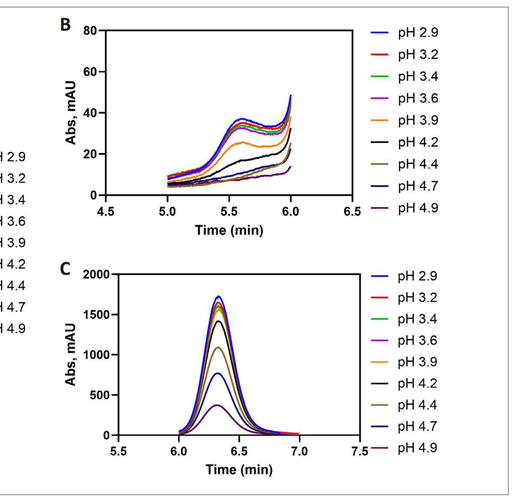

Figure 8: *Comparison of chromatography from Praesto™ Jetted A50 HipH purified mAb eluted at different pHs. A) Full chromatograph, B) Aggregate, C) Monomer.*

RESULTS

- Automated method established on the Tecan Fluent enables purification and buffer exchange of 96 samples in under 60 minutes.
- MabSelect™ PrismA has a higher binding capacity than Praesto™ Jetted A50 HipH.
- Using two different cell supernatant samples containing mAbs:
  - Both resins achieve similar recovery across a range of low pH values.
  - Comparable yields up to pH 3.6.
- At low pH elution conditions:
  - Monomer percentages of the two antibodies tested were notably influenced by the resin used even at the same elution pH.
  - As pH increases, monomer percentages increases.
- At higher pH elution conditions (3.9 and above):
  - Praesto™ Jetted A50 HipH resin yields significantly higher recovery.

CONCLUSION

- IMCStips allow for efficient optimization of protein purification allowing for streamlined testing of resin types, binding capacity, and elution conditions.
  - Traditional methods such as FPLC would require significantly more hands-on time.
- Data shows that recovery and monomer content are influenced by:
  - The specific resin used for purification.
  - The elution pH.
- This work paves the way for automating and optimizing antibody purification screening strategies to improve both yield and monomer content.

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