Analytical Scale 96-well Protein A Affinity Resin-Based Purification using Andrew+™ Automation Robot Supporting Upstream Bioprocessing[1]

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OVERVIEW

- Rapid and automated Protein A purification using Andrew+ Robot
- Automation protocol in OneLab library, readily available for download
- Dynamic protocol to cover number of samples from 1 to 96 in a well plate format.
- Robust protocol with excellent recovery and reproducibility.
- Excellent chromatogram characteristics of Waters BioResolve Protein A Affinity column for the quantification of monoclonal antibodies

INTRODUCTION

Affinity purification of recombinant monoclonal antibodies (mAbs) uses a binding agent to reversibly isolate antibodies from complex matrices such as harvested cell culture fluid (HCCF). In recombinant mAb production and analysis, Protein A (ProA) immobilized on a solid surface is the most commonly used affinity ligand. In biotherapeutic process optimization, both process- and product-related quality attributes are routinely monitored. Multi-parallel bioreactor systems and fed-batch DoE studies over 14 days can generate a large number of samples. To support efficient, time-course analysis of multiple product attributes, automated purification becomes critical to the workflow.

This work describes automated purification using the Andrew+ Robot and ProA resin for up to 96 HCCF samples in a 96-well format.[2] The protocol is dynamic: users enter the required sample number, and the system adjusts automatically. Recovery and repeatability were evaluated by LC-UV using a Waters BioResolve Protein A Affinity Column with immobilized ProA ligand.

METHODS

A commercially available NISTCHO cell line expressing mAb was cultured for 14 days to generate HCCF samples. Day 14 samples were pulled, centrifuged, and filtered through 0.2 µm syringe filter. Samples were stored in a -80 °C freezer.

The HCCF sample was transferred into a 96-well plate and mAb was purified using Andrew+ Robot. The purified mAb and unpurified mAb in HCCF were subject to LC-UV analysis with NISTmAb as the calibration standard. All samples were injected in triplicate.

	Description of Automated ProA Purification
1	Load ProA resin to filter plate, wash the resin with water and phosphate buffer
2	Add 120 µL HCCF solution to the filter plate
3	Shake the plate at 1250 RPM and 12 °C for 20 minutes using Eppendorf shaker
4	Wash resin-bound antibody with phosphate buffer and water
5	Add Tris neutralization buffer to receiving plate
6	Release antibody with 30 µL 0.1 M glycine three times. Incubate for 5 minutes each time.

Table 1. An overview of ProA purification automation steps using Andrew+ Robot.

LC Method Conditions

System:	ACQUITY™ Premier BSM LC-UV System	
LC Conditions:	Waters BioResolve Protein A Affinity Column, MaxPeak™ Premier, 2.1 mm x 20 mm (p/n: 186011369)	
	Mobile phase:	A: 1xDPBS B:0.1%Formic acid
	Column temp.	25 °C
	Sample temp.	8 °C
	Injection volume	2 µL
	UV Wavelength	280 nm
	Sampling rate	80 /s
	Run time (gradient):	3 minutes
LC-software	waters_connect™ Software	

Gradient Table

Time (min)	Flow Rate (mL/min)	Composition A (%)	Composition B (%)	Curve
0	0.75	100	0	Initial
0.5	0.75	100	0	6
0.51	0.75	0	100	6
1	0.75	0	100	6
1.01	0.75	100	0	6
1.20	0.75	100	0	6
1.21	0.75	0	100	6
1.40	0.75	0	100	6
1.41	0.75	100	0	6
1.60	0.75	100	0	6
1.61	0.75	0	100	6
1.80	0.75	0	100	6
1.81	0.75	100	0	6
3.00	0.75	100	0	6

The gradient wash cycle is added to minimize potential carryover.

RESULTS

The automated ProA affinity purification protocol used Cytiva MabSelect™ resin. A general description for the OneLab protocol is summarized in Table 1 and the Andrew+ Robot deck layout is shown in Figure 1A. The eluted mAb solution was neutralized to a pH 7.2 for protein stability and long-term storage.

The total recovered volume was 100 µL. The total time for the purification is 75 minutes for a plate of 96 samples. The protocol is dynamic, allowing it to purify any number of samples from 1 to 96 samples in a well plate. An example dialog box is shown in Figure 2B. It is recommended to enter samples as multiples of eight to maximize speed with the 8-channel pipette.

A)

B)

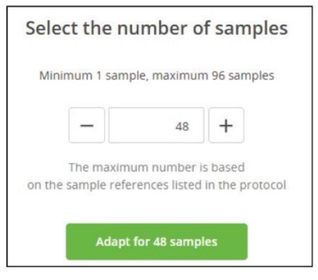

Figure 1. (A) Placement of Andrew+ Dominos for the ProA affinity purification of 96 samples. Position 1&2, 300 µL pipette tips; 3, QuanRecovery™ plate; 4, HCCF sample; 5, Extraction+ with filter plate; and 6, reagent trough. Users should follow OneLab screen instruction for the final placement. (B) Dialog box for dynamically specified number of samples.

Figure 2 shows TUV chromatograms and calibration curve of the NISTmAb standard solutions, ranging from 0.05–5 mg/mL, with linearity R^2 = 0.998 and %deviation <10%. The column produced excellent peak characteristics, 5x narrower peak width than other columns commonly used for titer determination.[3] The sharp peak produced by the column resulted in peak area %RSD <1% for all triplicate injections per well (Figure 3).

RESULTS

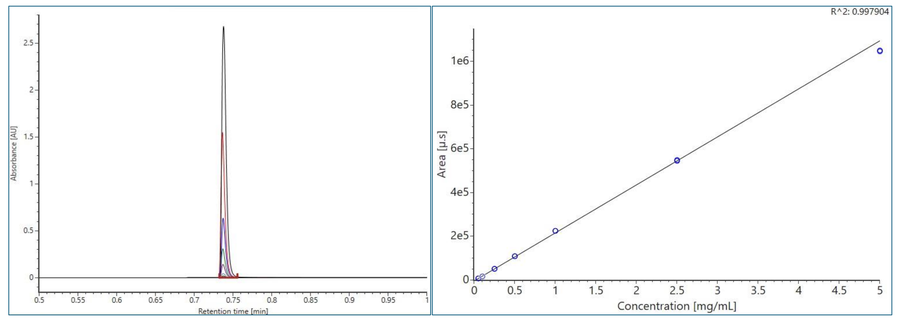

Figure 2. TUV chromatogram and calibration curve of NISTmAb standard solution with concentration ranging from 0.05 mg/mL to 5 mg/mL collected with LC-UV using Waters BioResolve Protein A Affinity Column.

%RSD	1	2	3	4	5	6	7	8	9	10	11	12
A	0.42%	0.07%	0.36%	0.10%	0.37%	0.18%	0.21%	0.10%	0.56%	0.11%	0.27%	0.28%
B	0.36%	0.08%	0.53%	0.06%	0.06%	0.31%	0.08%	0.57%	0.05%	0.09%	0.25%	0.04%
C	0.21%	0.05%	0.25%	0.02%	0.31%	0.31%	0.11%	0.50%	0.12%	0.04%	0.23%	0.07%
D	0.25%	0.04%	0.32%	0.11%	0.56%	0.20%	0.08%	0.37%	0.06%	0.06%	0.29%	0.03%
E	0.38%	0.09%	0.40%	0.06%	0.04%	0.30%	0.02%	0.20%	0.26%	0.07%	0.49%	0.26%
F	0.43%	0.11%	0.16%	0.23%	0.09%	0.46%	0.21%	0.07%	0.42%	0.27%	0.02%	0.41%
G	0.07%	0.34%	0.14%	0.05%	0.36%	0.04%	0.12%	0.15%	0.05%	0.17%	0.26%	0.10%
H	0.30%	0.12%	0.41%	0.28%	0.48%	0.01%	0.07%	0.46%	0.13%	0.04%	0.38%	0.08%

Figure 3. Peak area %RSD of triplicate injections for each of 96 samples analyzed by LC-UV using Waters Protein A Column.

mAb recovery was estimated from purified and unpurified HCCF samples. The determined concentration of mAb in purified sample was 1.68±0.14 µg/µL, with 8.5% RSD for 96 samples. The overall recovery for 96 samples was calculated to be 96.8±8.2%. Figure 4A shows the average mAb recovery obtained per well, and Figure 4B shows the histogram of the %recovery distribution. Overall, the automated purification using Andrew+ Robot is robust and highly reproducible.

A)

%RSD	1	2	3	4	5	6	7	8	9	10	11	12
A	109%	84%	86%	100%	112%	92%	92%	93%	91%	96%	87%	91%
B	98%	86%	90%	95%	94%	97%	83%	94%	85%	94%	98%	93%
C	100%	110%	95%	90%	91%	112%	96%	109%	93%	93%	88%	95%
D	100%	113%	109%	88%	108%	113%	117%	92%	103%	107%	105%	90%
E	102%	104%	92%	107%	108%	93%	92%	89%	88%	95%	91%	94%
F	104%	109%	92%	90%	109%	111%	106%	89%	95%	96%	102%	94%
G	93%	89%	106%	87%	107%	95%	88%	87%	92%	96%	84%	105%
H	97%	95%	100%	110%	94%	88%	82%	93%	93%	104%	99%	95%

B)

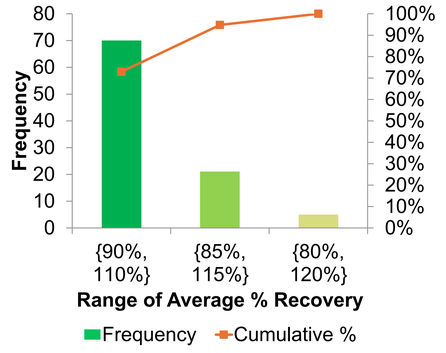

Figure 4. (A) Average mAb recovery per well in 96-well plate following automated ProA purification protocol, n=3. Wells are color-coded according to their value, with dark green values falling within 10% of 100% mAb recovery relative to the unpurified sample (full recovery); light green values falling within 15% of full mAb recovery; and pale yellow falling within 20% of full mAb recovery. (B) Distribution of average mAb recovery in the 96-well plate, following the same color scheme as (A). The cumulative count contributed by each range is represented as a percentage by the orange overlay line.

CONCLUSION

An automated ProA affinity purification protocol for 96 samples using Andrew+ Robot has been streamlined and uploaded to OneLab library. The protocol can be downloaded free of charge. The rapid purification of 120 µL HCCF resulted in 100 µL mAb solution, sufficient for multiple critical quality attribute (CQA) analyses.

References

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