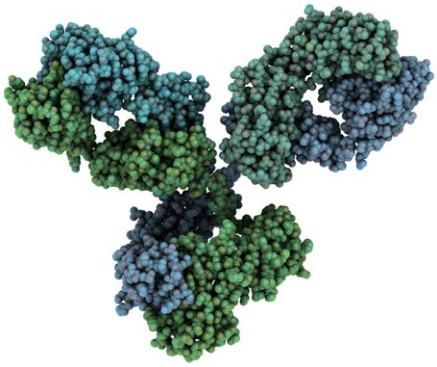
Science Together

KNAUER

Comparison of two column sets for antibody purification in an automated two step purification process



Ulrike Krop, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin;
www.knauer.net

SUMMARY

This application compares the automated purification of antibodies with the AZURA® Bio purification 50 – Two Step Purification System with different columns. Capacity and yield of the purified proteins were compared and revealed no significant differences in the performances of the two investigated column sets.

INTRODUCTION

Antibodies play an important role in the biotechnology and pharmaceutical industry. They are used in a variety of applications where quality and purity of the antibodies is crucial. The most widely used technique for antibody purification is protein A affinity chromatography. It is a very efficient capture step and delivers highly clean protein. The antibodies are eluted under acidic conditions requiring an additional buffer exchange step. This desalting step requires in many cases manual interaction. The AZURA Bio purification 50 – Two Step Purification System allows an automated purification without manual interaction. Various resins from different vendors are available for protein A affinity and desalting purification. The aim of this application was to compare different column sets for the affinity and desalting phase for this specific two step approach.

Comparison of two column sets for antibody purification in an automated two step purification process

RESULTS

Antibodies were purified from 100 µL reconstituted human plasma by protein A affinity chromatography and a subsequent buffer exchange step. The chromatogram of the whole purification process is divided in two steps **(Fig 1)** During the first step, the sample is injected. All non-binding proteins flow through the column (Peak A). Next, all remaining impurities are washed from the affinity column. Elution takes place under low pH conditions (Peak B1). The eluted sample was stored in a sample loop and reinjected in step two on a desalting column. Finally, the elution peak (Peak B2) was collected. In the chromatogram two example purifications with different column sets are depicted. The antibodies purified with the vendor X Protein A FF and Desalting columns (red signal) and Sepapure Protein A FF and Sepapure Desalting columns (blue signal) are comparable. An average of 0.37 ± 0.05 mg proteins was purified with the vendor X Protein A FF and Desalting column in comparison to an average yield of 0.41 ± 0.1 mg protein with Sepapure Protein A FF and Sepapure Desalting columns **Tab 1** suppl. Material). Finally, SDS-PAGE was performed to analyze the purity of the samples **(Fig 2)**.

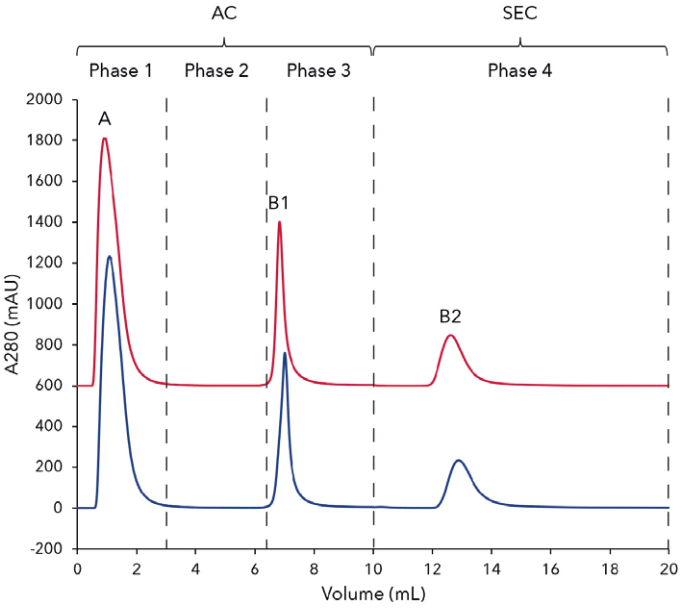


Fig. 1 Overlay of chromatograms of the two step antibody purification; Step 1 – Affinity chromatography (AC): Phase 1) Sample injection; Phase 2) Column washing; Phase 3) Elution of antibodies and parking in sample loop; Step 2 – Buffer exchange with desalting column: Phase 4) Elution of antibody; A – flow through of unbound protein; B1 – elution peak of antibodies from Protein A column; B2 – elution peak of antibodies from desalting column; Red signal : Purification with vendor X Protein A FF 1 mL and Desalting 5 mL column Blue signal: Purification with SepapureProtein A FF 1 mL and Sepapure Desalting 5 mL column

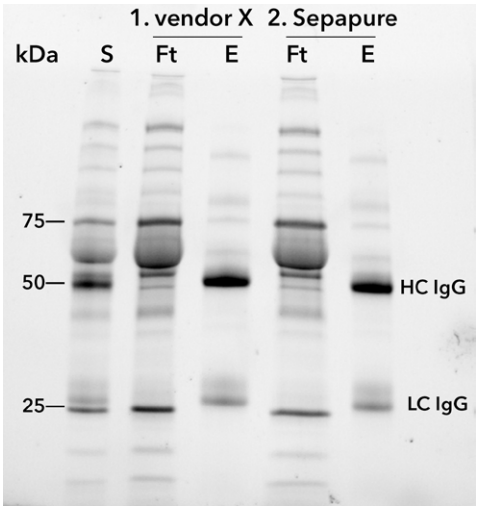


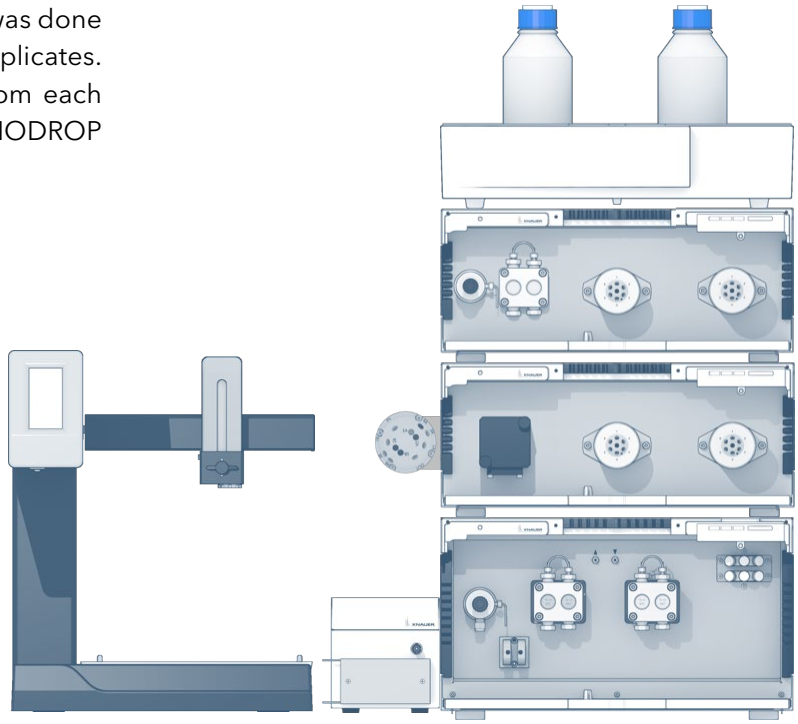
Fig. 2 SDS-PAGE at different purification steps; 1. Purification with vendor X Protein A FF 1 mL and Desalting 5 mL column, 2. Purification with Sepapure Protein A FF 1 mL and Sepapure Desalting 5 mL column, S) serum before purification; FT) flow through, E) eluted antibodies (IgG) heavy chain (HC) and light chain (LC) after two step purification

MATERIALS AND METHODS

In this application an AZURA Bio purification 50 – Two Step Purification System was used. It consisted of an AZURA P 6.1L 50 mL HPG metal-free pump, 1st AZURA ASM 2.1L assistant module with 50 mL feed pump and two 6 port/2 position valves, 2nd AZURA ASM 2.1L with UVD 2.1S and two 6 port/2 position valves, column switching valve; conductivity monitor and a fraction collector. The protein A and Desalting columns were equilibrated with buffer A (20 mM Sodium Phosphate Buffer, pH 7.4). The flowrate for the 1 mL protein A columns was 1 ml/min and for the 5 mL Desalting columns was 5 mL/min. 100 µL of reconstituted human plasma was injected on to the protein A column. The column was washed with buffer A. Antibodies were eluted with buffer B (0.1 M Glycin-HCL, pH 2.7). Via a threshold function the elution peak was parked in a 5 mL sample loop. Subsequently, the eluted protein was re-injected on to the desalting column for buffer exchange with buffer A. The eluted antibodies were collected with the fraction collector. The UV signal was measured at 280 nm and conductivity signal was recorded. Each purification was done with two column sets from each vendor in triplicates. The concentrations of the eluted protein from each individual run were determined with a NANODROP 2000 and analyzed by SDS-PAGE.

CONCLUSION

The AZURA Bio purification 50 - Two Step Purification System was used to analyze the automatic purification of human antibodies with two different sets of columns from different vendors. The yield and purity of the eluted antibodies was for both column sets comparable. With the two step purification system, no manual interaction was necessary between the first protein A affinity chromatography step and the second buffer exchange/desalting step. This setup can be adapted to other purification protocols and can be used for a variety of materials. In conclusion, the purification is quantitatively and qualitatively identical for both tested column materials in the two step system setup. The tested column column materials are suitable for two step purification.



ADDITIONAL RESULTS

Tab. A1 Yield of the purified antibodies

Column set	Repetition	Protein A Column	Desalting column	Yield in mg	Mean
1	1	vendor X Protein A FF 1 mL	vendor X Desalting 5 mL	0.38	0.37 ±0.05
	2			0.40	
	3			0.45	
2	1			0.33	
	2			0.32	
	3			0.36	
1	1	Sepapure Protein A FF 1 mL	Sepapure Desalting 5 mL	0.63	0.41 ±0.10
	2			0.38	
	3			0.38	
2	1			0.34	
	2			0.34	
	3			0.41	

ADDITIONAL MATERIALS AND METHODS

Tab. A2 Method parameters

Eluent A	20 mM Sodium phosphate buffer pH 7.4		
Eluent B	100 mM Glycine, pH 2.7		
Gradient	Volume [mL]	% A	% B
AC Injection+Wash	0-5	100	0
AC Elution	5.02-10	0	100
SEC/Desalting	10.02-20	100	0
Flow rate	1 mL/min (Protein A) 5 mL/min (Desalting)	System pressure	>3 bar
Run temperature	RT	Run time	12 min
Injection volume	100 µL	Injection mode	Injection valve
Detection UV	280 nm	Data rate	2 Hz

Tab. A3 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L, HPG 50 mL pump head, ceramic	APH68FB
Assistant 1	AZURA ASM 2.1 L Right: 6 Port 2 Pos valve 1/16", PEEK Middle: 6 Port 2 Pos valve 1/16", PEEK Left: P 4.1S, 50 mL pump head, ceramic	AYBLECEC
Assistant 2	AZURA ASM 2.1 L Right: 6 Port2Pos valve 1/16", PEEK Middle: 6 Port2Pos valve 1/16", PEEK Left: UVD2.1S	AYCAECEC
Flow cell	3 mm path length, 1/16", 2 µL volume, 300 bar, biocompatible	A4045
Conductivity monitor	AZURA CM 2.1S	ADG30
Flow cell	Preparative up to 100 mL/min	A4157
Column	Sepapure Protein A FF 1ml Sepapure Desalting 5 ml vendor X Protein A FF, 1ml vendor X Desalting, 5 ml	010X40USPZ 020X46OSPZ
Fraction collector	Foxy R1, microplates rack	A59100
Software	Purity Chrom Basic	A2680

RELATED KNAUER APPLICATIONS

VBS0063 – Automated two – step purification of mouse antibody IgG1

VBS0064 – Comparison of IgG purification by two different protein A media

VBS0067 – Automated two step purification of 6xHis-tagged GFP

VBS0070 – Ion Exchange Chromatography with AZURA® Bio purification system

