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Application Highlight: Automated two-step purification of antibodies

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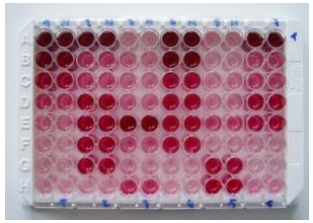
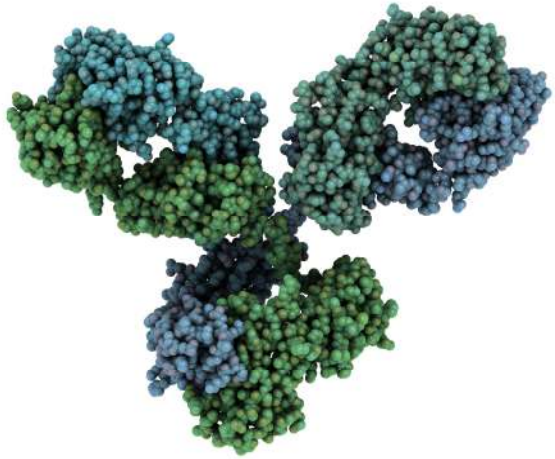
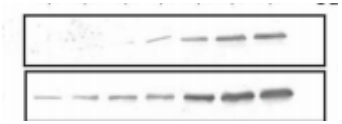
Why antibodies?







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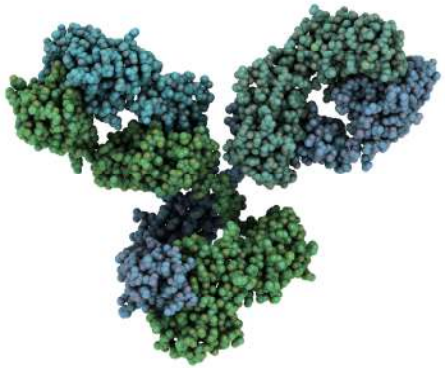
Antibodies



Monoclonal Antibodies

Polyclonal Antibodies

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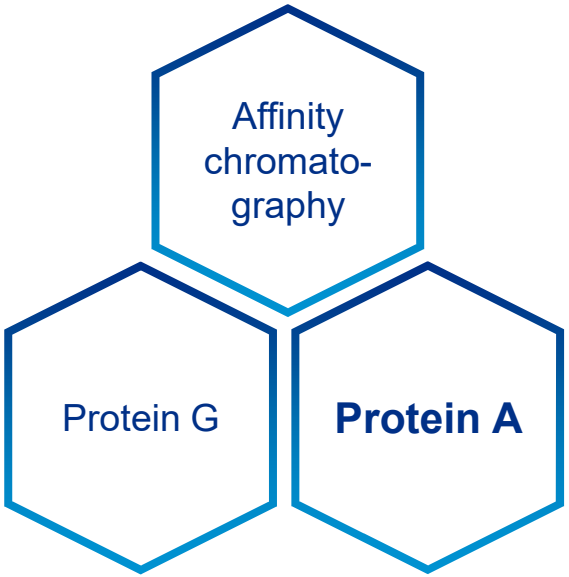
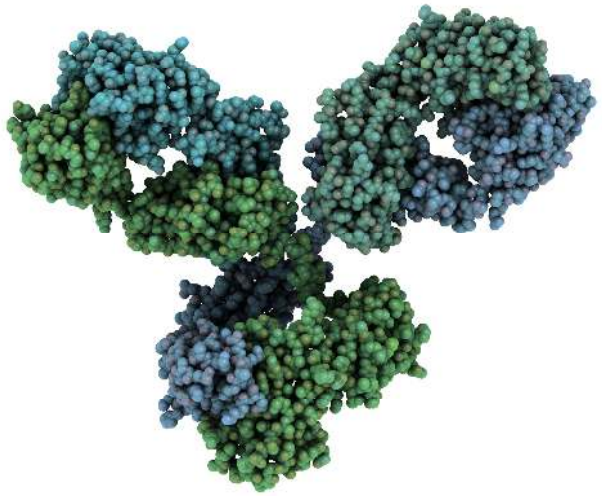
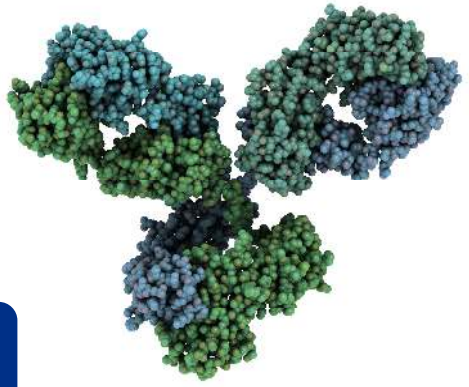
Antibody purification





Antibody purification



AC: Protein A → Elution: Acidic pH → SEC: Buffer exchange

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Antibody purification protocol

Approach

- Affinity purification – protein A column
- pH change (pH3 – pH7) by buffer exchange
- Ready to use antibody

Conditions

Eluent A:	Washing buffer: TBS (Tris-buffered saline)
Eluent B:	Elution buffer: 0.2 M Na-Citrate, pH 3
Eluent C:	Storage buffer: PBS (phosphate buffered saline) pH 7.4
Flow rate:	1 mL/min
System pressure:	1.5 bar
Temperature:	RT
Run time:	55 mL
Injection volume:	10 mL
Injection mode:	Feed pump
Detection:	280 nm (UV)

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Automation with Two-Step Purification

"You can automate your purification and safe time and resources. Automation by combining two methods increases the efficiency and optimizes the workflow. "

Yannick Krauke / Application Specialist

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2-step Automated Purification System

Save time

Save manpower

Minimize human error

Higher reproducibilty

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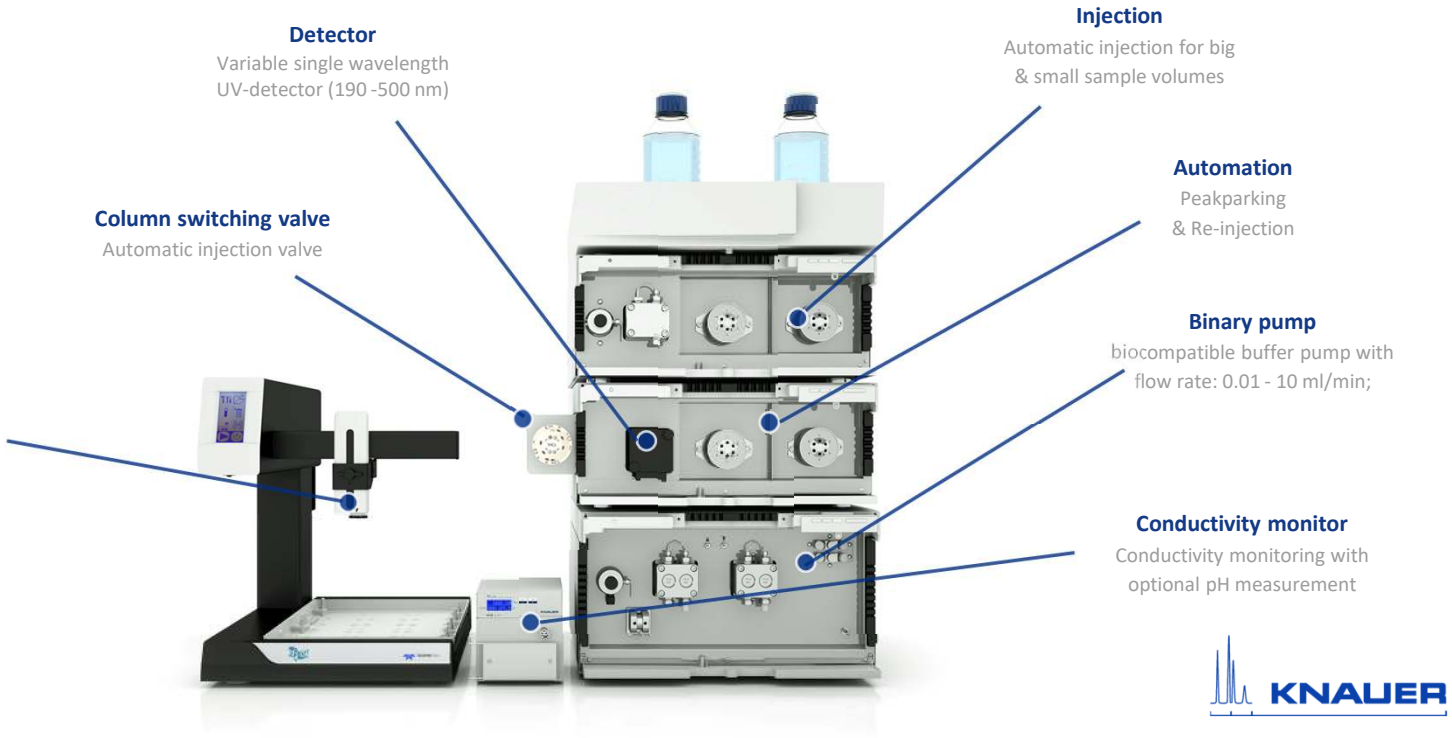
2-step Automated Purification System



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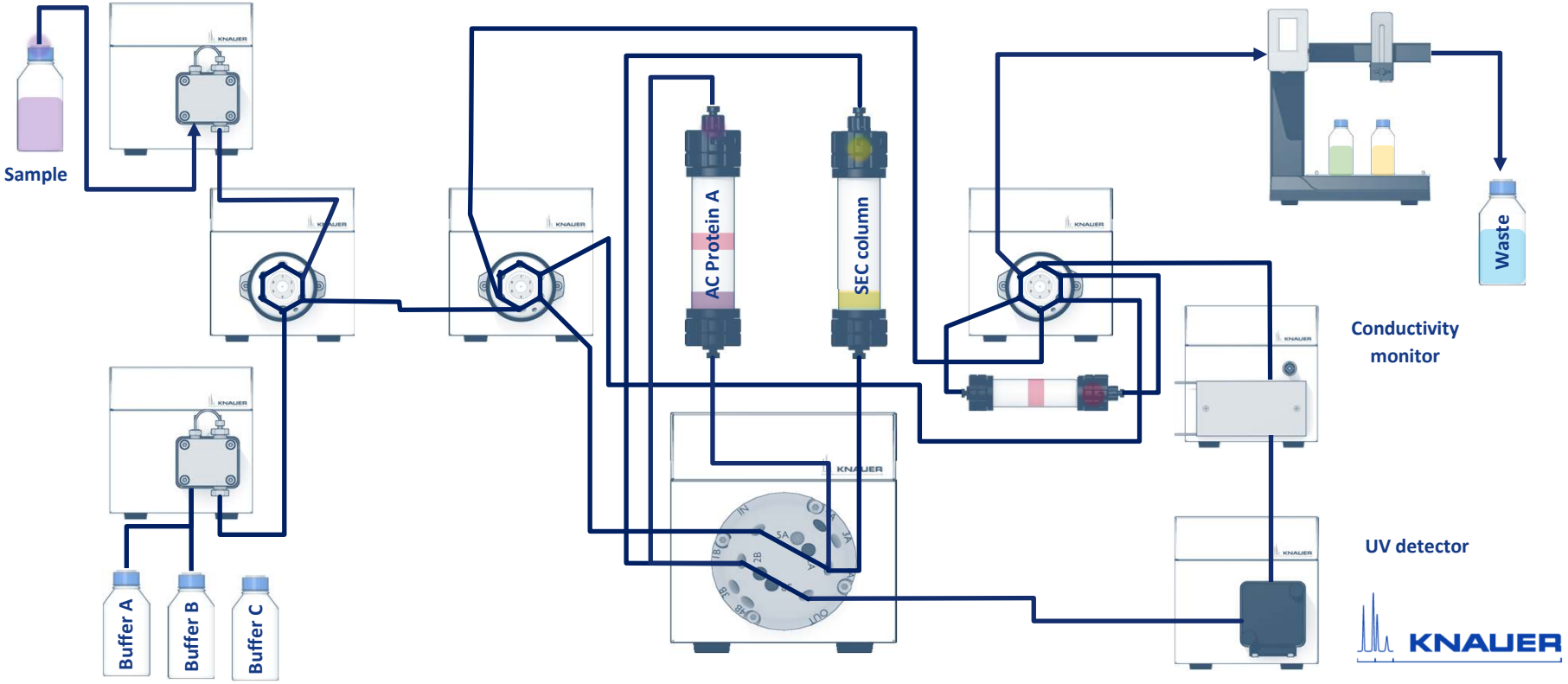
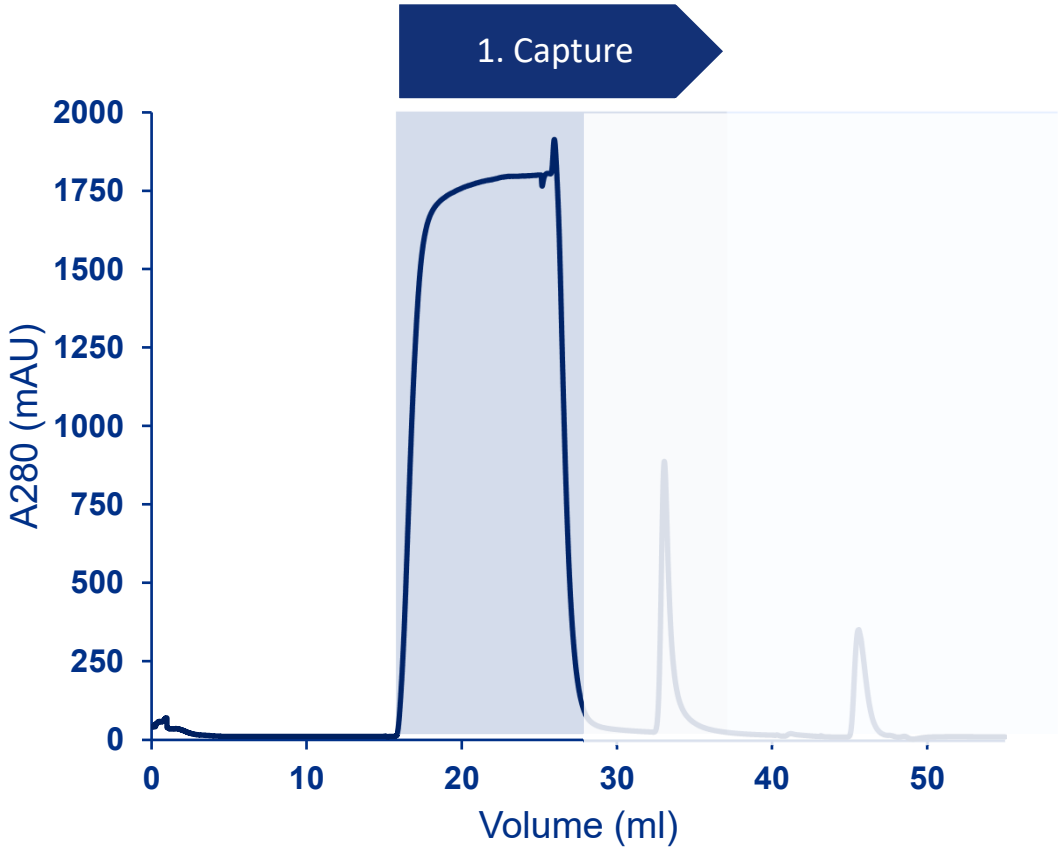
2-step Automated Purification System

Schematic overview of two step purification of mouse antibody



Sample application & column wash



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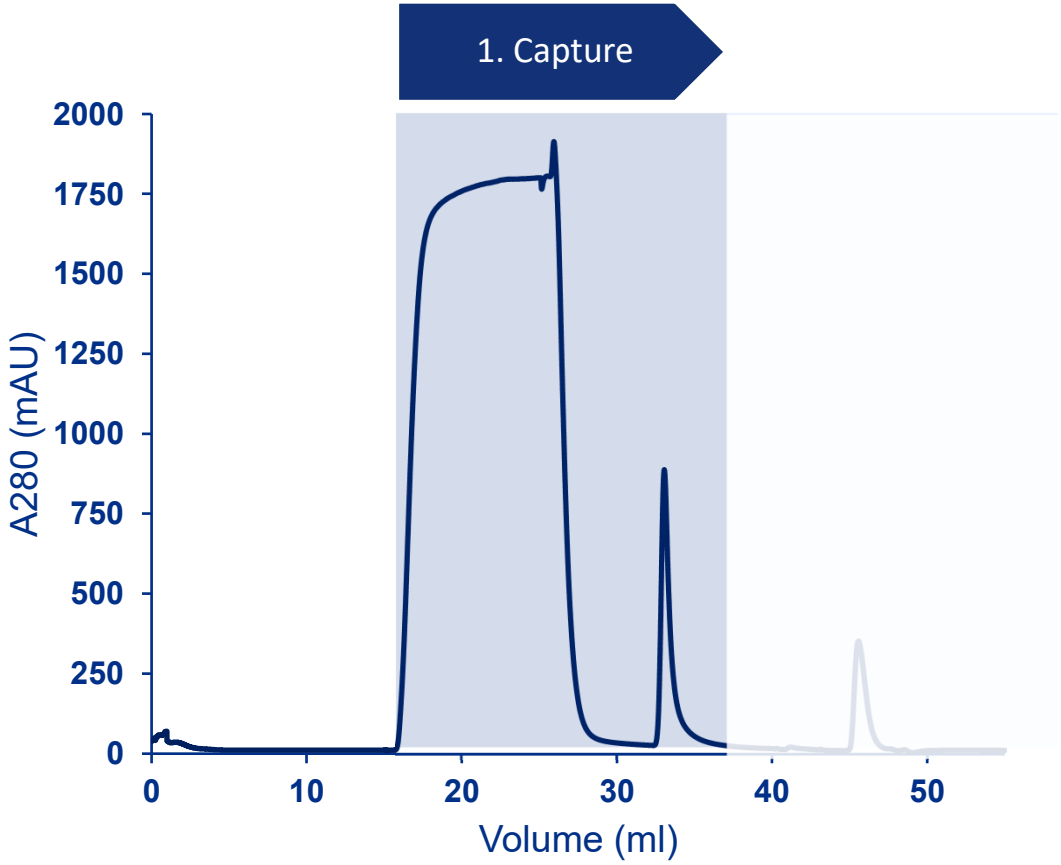
Elution of IgG



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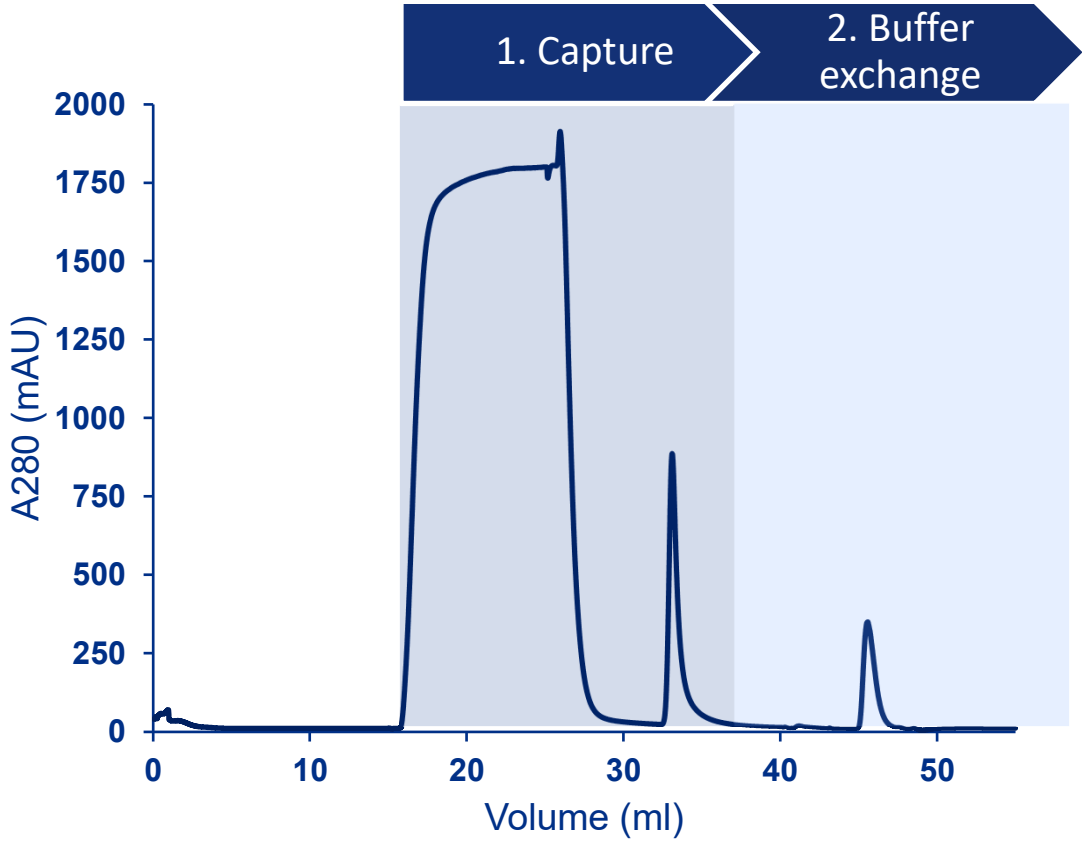
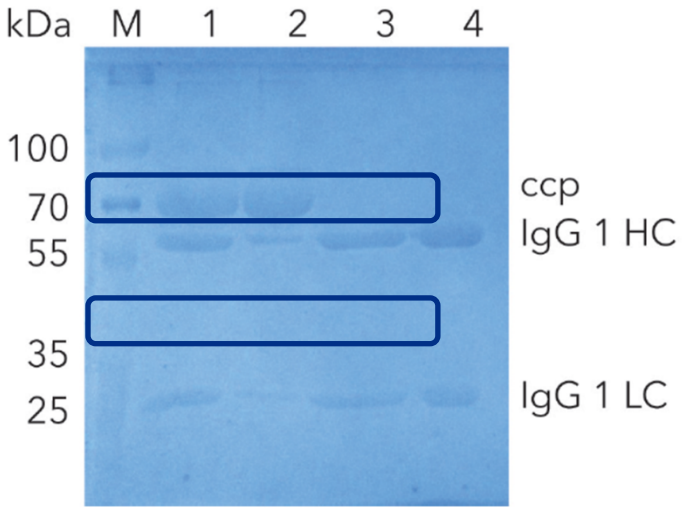
Buffer exchange with desalting column



Two-step purification



No protein loss in 2nd purification step

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AUTOMATED TWO - STEP PURIFICATION OF MOUSE ANTIBODY IGG1 WITH AZURA BIO LC LAB SYSTEM

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

SUMMARY

This application highlights the possibility of automated purification of antibodies (IgG) with the AZURA® BIO LC system without manual interaction during purification process. The cell culture was applied with a feeding pump on a proteinA affinity column to capture and purify the antibodies. These were kept in the system and in a second step applied on a gel filtration column for buffer exchange.

INTRODUCTION

Antibodies (immunoglobulins, Ig's) are part of the immune system. They can identify and bind particular antigens thereby neutralizing them. Due to their specific target recognition/binding function they have a significant importance in the biotechnology and pharmaceutical industry. Key applications are the diagnosis and treatment of diseases. Besides, antibodies are also the crucial components in numerous research applications such as Western Blots and immunoassays. Quality and purity of the IgG is crucial for these applications. The purification of antibodies involves two to three steps, 1. capture step, (2. intermediated step), 3. polishing step. The transition from one to another step generally involves manual interaction and thus is time consuming.

The aim of this application note was to establish an automated purification method on the AZURA Bio LC purification system combining an affinity chromatography step with a gel filtration step to exchange the buffer of the purified antibodies.

RESULTS

The mouse immunoglobulin (IgG1) was purified from 10 mL cell culture by affinity chromatography, using a by proteinA column. The chromatogram of the IgG purification shows the four main steps of the procedure (**Fig.1**). Step 1: equilibration of the proteinA column with buffer A . Step 2: sample injection by the feed pump. The large flow through peak (**A**) visualizes the cell culture matrix and proteins not bound by the proteinA column. Subsequently, the column was washed with buffer A until no further peaks were detected. Step 3: elution of the captured IgG1 with buffer B and parking in the sample loop (**B1**). Step 4: immediate buffer exchange was performed by the flushing of the system with exchange buffer C and the following re-injection of the IgG1 on the gel filtration column. The eluting peak was recovered by the fraction collector (**B2**). The main aim of the second step was the buffer exchange shifting the pH from 3 to 7. The conductivity signal was recorded, demonstrating the desalting of the eluates during the purification process (**Fig. 2**).

Finally, a SDS-PAGE was performed to control the result of the purification steps (**Fig. 2**). The analysis of the flow through and comparison with the injected sample show that some IgG1 did not bind to the protein A column (lane 1 and 2). The protein bands of IgG1 heavy chains (HC) and IgG1 light chains (LC) are visible at 55 kDa and 22 kDa in the SDS-PAGE. Further, a larger un-specific band at 70 kDa was detected. This band was only detected in the flow through and not in the purified IgG (lane 2, 3, 4) showing that IgG1 was not contaminated with other proteins. The IgG1 after the proteinA column (lane 3) and the proteinA / gel filtration column (lane 4) have a similar concentration showing no protein loss in the second purification step.

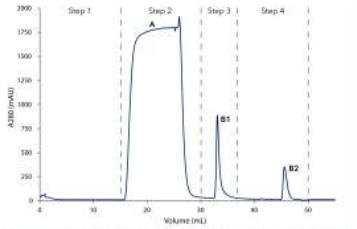

Fig. 1 Chromatogram of the two-step mouse IgG purification: Step1 - Affinity chromatography (AC): 1 Column equilibration with buffer A; 2 Feed injection and column washing; 3 Elution of IgG from proteinA column with buffer B and parking in 1mL sample loop; Step2 - Buffer exchange with desalting column: 4. Elution of IgG with buffer C; A-flow through; B1-elution peak of IgG from proteinA column; B2-elution peak of IgG after SEC desalting column

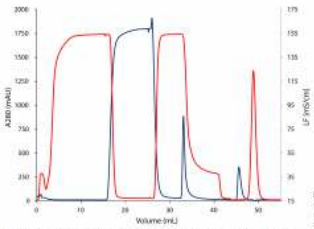

Fig. 2 Chromatogram of the two step mouse IgG purification with UV and conductivity signal; UV signal at 280 nm in blue; conductivity signal in red; after desalting (45min) elution peak (blue) and salt peak (red) are clearly separated

Fig. 3 SDS-PAGE of IgG1 at different purification steps; M: Marker (3 µL) in kDa; 1-serum (15 µL); 2-flow through (15 µL); 3-IgG1 pure (15 µL) after elution from protein A column, intermediate step; 4-IgG1 pure (15 µL) after protein A and desalting column; ccp: cell culture proteins; IgG1 HC (heavy chain); IgG1 LC (light chain)

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Application areas

- Affinity chromatography for any „tagged“ protein (e.g. GST)
- IMAC (immobilized metal ion affinity) for His-tagged proteins
- Antibody purification – protein A, G, L columns
- IEC

- Gel filtration, buffer exchange – reducing salt concentration, pH change
- IEC

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Another example:
Purification of 6xHis-tagged GFP

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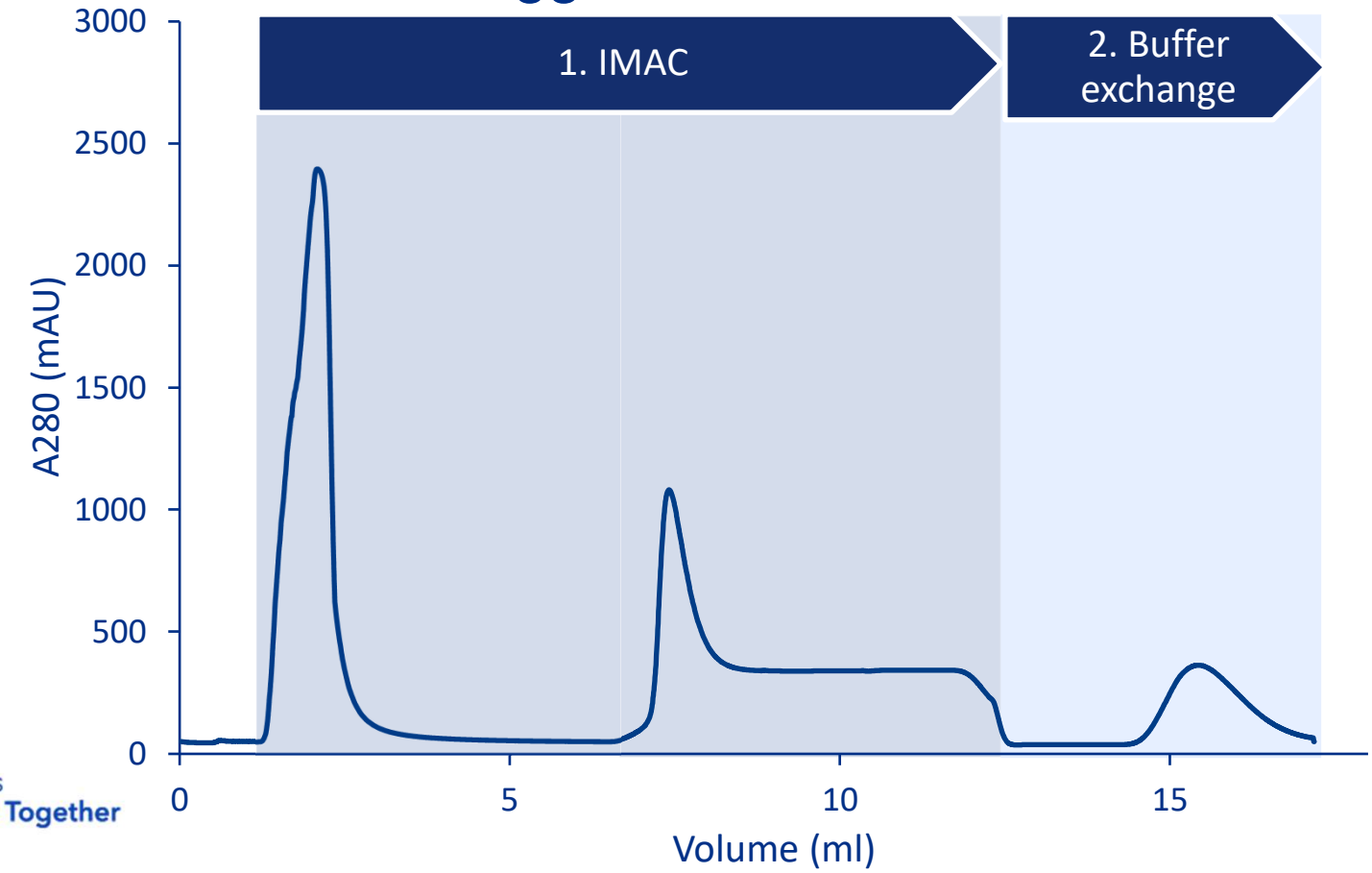
Purification of 6xHis-tagged GFP



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AUTOMATED TWO-STEP PURIFICATION OF 6xHIS-TAGGED GFP WITH AZURA BIO LC

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

SUMMARY

Affinity chromatography by His-Tag is one of the most widespread purification techniques for recombinant proteins. In most cases it requires an additional cleaning/polishing step. This application highlights the possibility of combining two subsequent chromatography protocols without manual interaction using the AZURA Bio LC system.

INTRODUCTION

Affinity chromatography (AC) is one of the most efficient techniques to purify recombinant proteins. Mostly, AC is performed on crude samples like bacterial lysates containing the recombinant protein that is genetically engineered to be expressed with a tag that enables the specific capture of the recombinant protein. These highly efficient tags are used for affinity binding to specific affinity chromatography materials. A variety of tags is available among which the polyhistidine tag is the most widespread one. In this application, six histidine (6xHis) residues were attached to the green fluorescent protein (GFP). The histidine residues bind with very high affinity to the immobilized metal ions on the column ("immobilized metal ion affinity chromatography / IMAC). In many protocols, an additional step is recommended to reach higher purity or to change the buffer of the purified protein to a suitable storage buffer. Here, size exclusion chromatography was used as second step to exchange the buffer of the purified protein. Purification of recombinant proteins can be performed manually or by using a chromatography system combining two steps automatically to save time and effort.

RESULTS

The chromatogram of the 6xHis-GFP purification shows the five phases of the two-step protocol (**Fig. 1**). After equilibration (**Fig. 1**, phase 1) the lysate was injected and the GFP bound to the Ni-NTA affinity column via the 6xHis-tag. All other non-binding proteins and impurities are in the large flow through peak (**Fig. 1**, phase 2, peak A). Subsequently, the column was washed until the baseline was stable (**Fig. 1**, phase 3). The eluted protein (**Fig. 1**, phase 4, peak B1) was collected in a sample loop and re-injected on the desalting column (**Fig. 1**, phase 5) to exchange the buffer from high imidazole concentrations to a buffer without imidazole. The purified protein (**Fig. 1**, peak B2) was collected by the fraction collector.
Additionally to the unspecific photometrical detection of all proteins at 280 nm, GFP-signal was recorded at 395 nm (**Fig. 2**) with the multi- wavelength detector. Most of the 6xHis-tagged GFP bound to the column as only a small peak for GFP is visible in the flow through. The purification results were confirmed by SDS-Page (**Fig. 3**). The cell lysate (**Fig. 3**, lane 1) shows a prominent band representing the overexpressed 6xHis GFP. This band is cleared in the flow through (**Fig. 3**, lane 2), confirming that most of the tagged protein bound to the column. The eluted sample (**Fig. 3**, lane 3) shows the purified 27 kDa 6xHis-GFP with only minor contaminations.

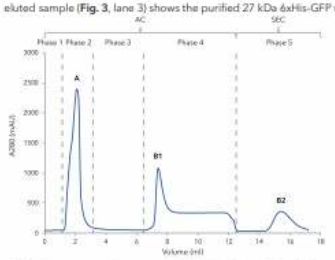

Fig. 1 Chromatogram of the two-step 6xHis-GFP purification; 280 nm UV signal; Step1 - Affinity chromatography (AC)/ Ni-NTA column: 1 Column equilibration; 2 Sample injection; 3 Column washing; 4 Elution of 6xHis-GFP and parking in 1ml sample loop; Step2 - Buffer exchange with desalting column: 5 Elution of 6xHis-GFP ; A - flow through of unbound protein; B1 - elution peak of 6xHis-GFP from Ni - NTA column; B2 elution peak of 6xHis-GFP

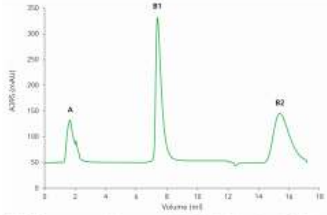

Fig. 2 Chromatogram of the two-step 6xHis-GFP; GFP detection with 395 nm UV signal, A - flow through of unbound protein; B1 - elution peak of 6xHis-GFP from Ni - NTA column; B2 elution peak of 6xHis-GFP

Fig. 3 SDS-PAGE of two-step 6xHis-GFP purification M: Marker, 1 - lysate before purification, 2 - flow through, 3 - eluted 6xHis-GFP (27 kDa) after two-step purification

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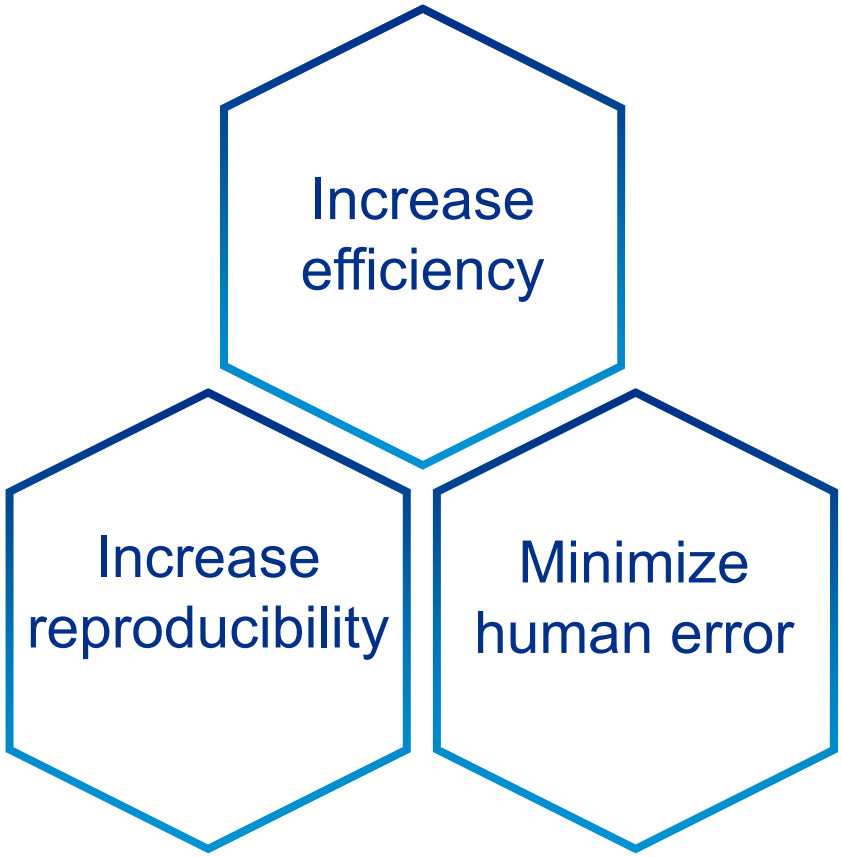
KNAUER FPLC: Automate your purification



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Configured AZURA Bio LC systems

TWO STEP PURIFICATION

AZURA COMPACT BIO LC SEC

AZURA COMPACT BIO LC AC

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