GE Healthcare
Life Sciences

Performing unattended, two-step protein purification with ÄKTA™ pure

A case study

Case: Performing unattended, two-step protein purification with ÄKTA™ pure

Purification of protein to a sufficiently high yield and purity can be a time- and resource-consuming process. The protein production group of Lou Fabri, Director at the Department of Protein Technologies, CSL, Melbourne, Australia processes as many as 1500 proteins per year. The goal is to obtain sufficient amounts of high-quality protein for further research studies. Study requirements can vary from a few to several hundred of milligrams of the target protein. The challenges include fast and efficient processes. One solution is a system that can handle different protein amounts and throughputs in, at least, two-step purification setups.

ÄKTA pure is a flexible chromatography system that allows you to automate both single- and multi-step purifications, depending on your needs. This case study describes how Lou Fabri used ÄKTA pure to purify and neutralize antibodies through an automated, two-step purification process, with high recovery and reproducibility.

Flexible
Different hardware modules can be selected depending on the functional needs for a specific application

Intuitive
Using UNICORN software it is easy to drag, drop, and edit phases to create preferred methods

Reliable
ÄKTA pure enables highly reproducible automation of either single- or multi-step purifications

Materials and methods

System configuration

In addition to core modules, such as A and B pumps, pressure monitor, mixer, injection valve, and conductivity monitor, ÄKTA pure 25 was configured with the modules listed in Table 1. Figure 1 shows the system setup in a schematic way.

Table 1. List of modules used for configuration of ÄKTA pure, in addition to core modules of the system

Module	Function
V9-A, V9-B	Gives 14 inlets for buffers and samples. Up to seven inlets can be used for sample loading. Integrated air sensors protect system from air.
V9-M	Mixer bypass valve that enables loading of samples with maintained integrity using the system pump. Will direct peak proteins to loop valve after elution.
V9-L	Loop valve holding up to five large loops (five 10 ml loops were used in this setup).
V9-C	Column valve for up to five columns.
U9-M	Multi-wavelength UV detector.
V9-O	Outlet valve. One of the outlets is connected to the mixer bypass valve to allow reinjection.
F9-R	Fraction collector to collect target protein fractions.

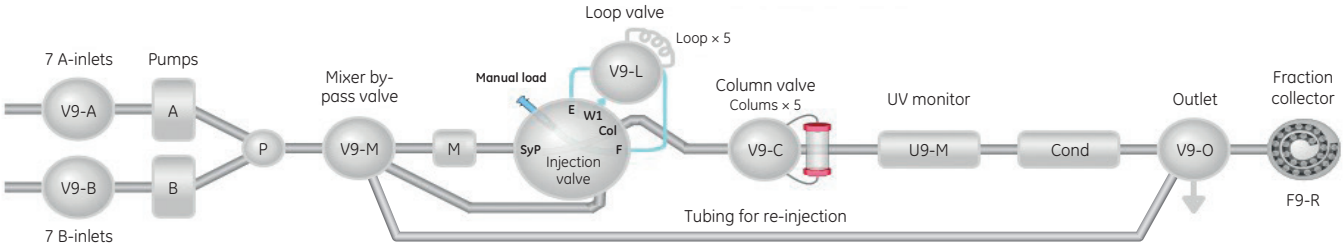


Fig 1. Schematic overview of the system setup. P = pressure monitor, M = mixer, Cond = conductivity monitor.

Process methods

For a fully automated process, a two-step method was created in UNICORN™ system control software and executed using the method queue functionality as outlined in Figure 2.

Part I: affinity capture and loop collection

Part II: neutralization through buffer exchange

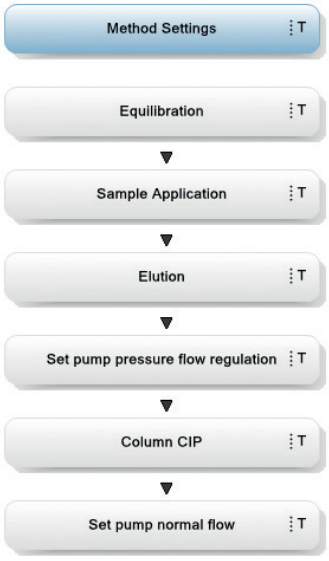


Fig 2. Outline of the automated two-step method. Part I gives an overview of the first purification step and includes column cleaning, preparation, sample loading, wash, and elution. Part II summarizes the second purification step including loading of the loop content onto the second column, collection of the purified and neutralized protein using the fraction collector, and cleaning of the second column. CIP = cleaning in place.

Sample, columns, and buffers

To evaluate the system setup and the efficiency of the ÄKTA pure two-step process, a purified IgG antibody was used as test sample. A sample mixture was prepared by dissolving IgG (1 mg/ml) in phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4) containing BSA (1 mg/ml).

A 1 ml HiTrap™ MabSelect SuRe™ column was used in the first purification step. The column was cleaned with 5 column volumes (CV) of 0.5 M NaOH followed by 5 CV of water, and equilibrated with 5 CV of PBS. A 5 ml sample was loaded via the buffer A inlet. After wash with PBS to remove all unbound material, proteins were eluted with 25 mM citrate buffer, pH 3.5.

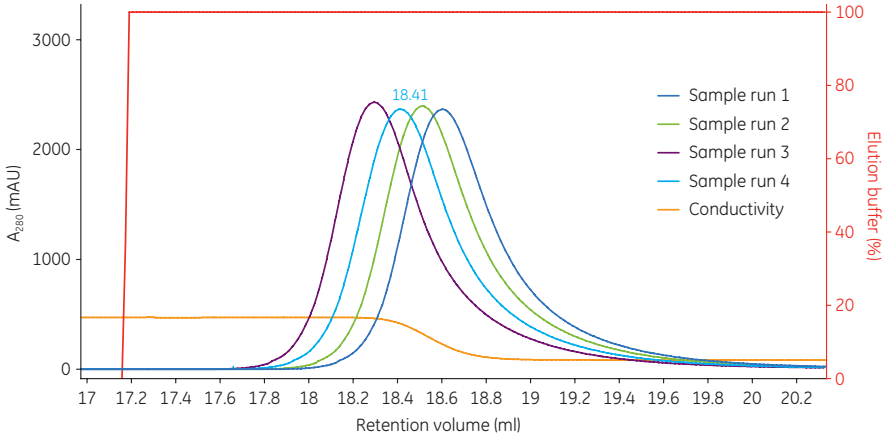
For the second purification step, a HiPrep™ 26/10 Desalting column was used. The column was cleaned with 0.2 CV of 0.5 M NaOH and equilibrated with 3 CV of PBS.

Samples were run in quadruplicates and applied through different loading tubes on the buffer A inlet valve. Eluted sample from the first chromatographic step was transferred to the holding loop, before quickly loaded onto the equilibrated desalting column to neutralize the sample. PBS was used as running buffer. Fractions of 10 ml were collected from the second purification step.

Results and discussion

For evaluation of ÄKTA pure system performance in an unattended, two-step purification application, the test purification was performed in four consecutive experiments. When comparing the separate runs, highly reproducible results were observed. Figure 3 displays an overlay of the chromatograms from the four consecutive runs in the first purification step. The variance in the peak area, which corresponds to protein amount, is only 0.07% between the runs. The consistency in peak area indicates a robust pump performance. As expected, the shape of the curves and the retention volumes are also similar between the runs.

Column: 1 ml HiTrap MabSelect SuRe
Sample: IgG 1 mg/ml in PBS, BSA 1 mg/ml, 5 ml
Flow rate: 1 ml/min
Wash: PBS
Elution buffer: 25 mM citrate buffer, pH 3.5
System: ÄKTA pure 25



Sample run	Retention volume (ml)	Peak area (ml × mAU)	Peak height (mAU)
1	18.60	1368	2366
2	18.51	1381	2394
3	18.30	1370	2429
4	18.41	1379	2366

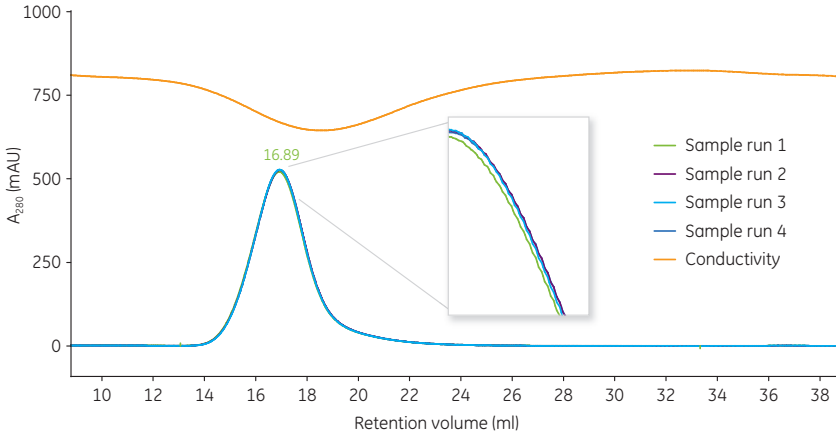
Fig 3. Overlay of the four chromatograms from Part I of the purification process. Note: The peak area determination in the graph is not linearly correlated to the total protein concentration as the UV readings are above the linear range 0 to 2000 mAU.

The large number of inlets enables running and cleaning of all columns used, as well as loading of up to seven different samples without reconfiguration of the system. The results show that a set sample volume gives the same outcome between runs even though samples are loaded through different inlet lines.

Figure 4 shows an overlay of the four chromatograms from the second purification step. The UV curves are close to identical as the peak elution volume was almost the same from each run. The protein recovery was shown to be over 90% as determined by spectrophotometric analysis (not described here).

The use of a mixer bypass valve and a loop valve facilitates intermediate storage of eluted protein from one column in a loop prior to reinjection onto the subsequent column (Fig 5). The automated, two-step purification procedure enabled a quick change of the low-pH elution conditions in the first purification step to more neutral conditions in the second step of the purification process. The ability to rapidly neutralize the sample pH can be critical for pH-sensitive target proteins.

Column: HiPrep 26/10 Desalting column
Sample: The collected peak from step one (stored in the loop valve)
Flow rate: 10 ml/min
Running buffer: PBS
System: ÄKTA pure 25



Sample run	Retention volume (ml)	Peak area (ml × mAU)	Peak height (mAU)
1	16.89	1403	521
2	16.95	1414	526
3	16.93	1404	527
4	16.95	1411	525

Fig 4. Overlay of the four chromatograms from Part II of the purification process.

Fig 5. The loop holder, used for intermediate peak storage, can for example be placed using the left side rails of the ÄKTA pure system.

Conclusions

This case study describes the use of ÄKTA pure chromatography system in an unattended, two-step purification process. The study shows the robustness of the system performance in an automated setup. The protein recovery achieved in the process was more than 90%.

Automation increases efficiency for researchers with numerous proteins to purify. ÄKTA pure can be configured to perform automated, unattended, single- or multi-step purification processes, including a variety of chromatographic techniques. The versatility of the system allows the use of one system instead of two to perform work similar to the case described here, meaning smaller footprint on the bench, which in turn means greater efficiency and optimization of the workflow. The quick and automated linkage of multiple chromatographic purification steps into one method eliminates manual sample handling and minimizes time spent between steps. As the system can be configured to handle several samples in one run, more proteins can be purified with the system at any given time.

ÄKTA pure is available in two versions, ÄKTA pure 25 and ÄKTA pure 150, supporting separate flow rates ranges. The system supports a wide range of chromatographic techniques and can be precisely tailored to suit a broad range of purification applications. ÄKTA pure chromatography system meets the automation requirements needed to deliver high recovery and reproducibility. This system is a valuable tool for researchers wishing to meet purification challenges, today as well as in the future.

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