Tandem two-step purification using **ÄKTA** pure or **ÄKTA** avant Cue Card

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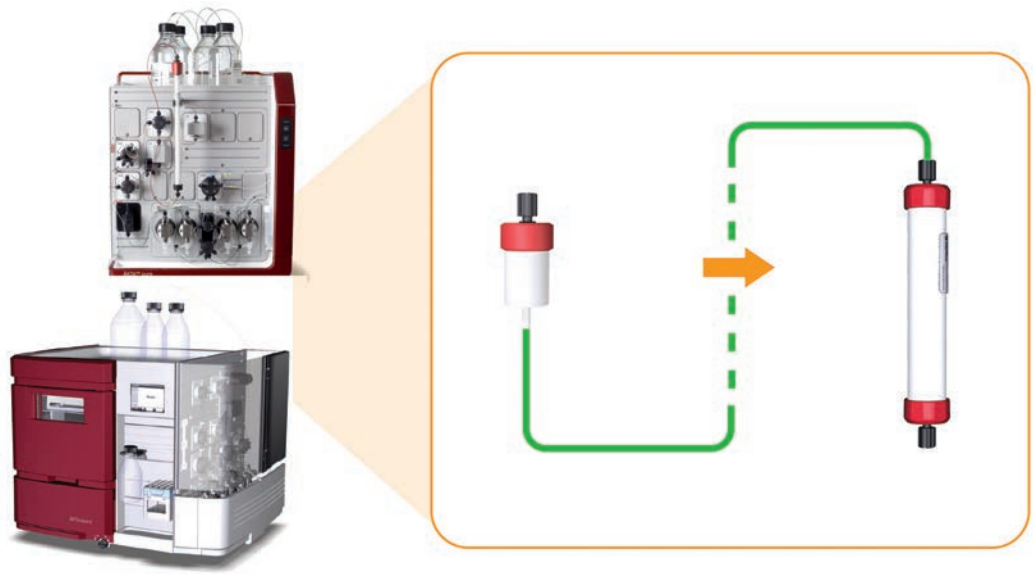


Introduction

This cue card describes how to configure ÄKTA™ pure, set up methods, and perform a fully automated two-step purification using two one-step UNICORN™ methods in a method queue. ÄKTA avant can be configured applying the same principle. The fully automated method is suitable for fast buffer exchange, for example after eluting the first column with low pH.

Example methods for two-step purifications can be downloaded from *cytiva.com/AKTA,* select ***ÄKTA pure*** or ***ÄKTA avant*** link under the heading ***Featured Products.*** Then click on ***Related Documents*** tab and find the heading ***Cue Card***.

The purpose of the cue card is to help users get started, and to inspire further two-step method development.

Principles

Two-step purification using a method queue with two one-step methods

By using one method for each purification step, column information from UNICORN can easily be used in the method. This means, for example, that pressure and flow rate limits are correct for each column and that column log book features can be utilized if preferred. Using a method queue allows full automation.

Method queue outline

Method 1: Affinity and peak elution to column two

↓

Method 2: Desalting or Gel filtration/Size exclusion

Method one

The user defined phase in method one defines all functionality for peak detection and redirection of the eluted peak to column two.

Method two

The redirected sample peak on column two is eluted in a desalting or gel filtration/size exclusion method.

Setup for tandem two-step purification

System configuration

Several different ÄKTA pure and ÄKTA avant configurations can be used. ÄKTA pure 25 is used in the following example. To enable tandem multi-step functionality, two Versatile valves **V9-V** and a Column valve (**V9-C** or **V9-Cs**) will be needed.

See the description below and the illustration for how to connect the modules.

Flow path connections:

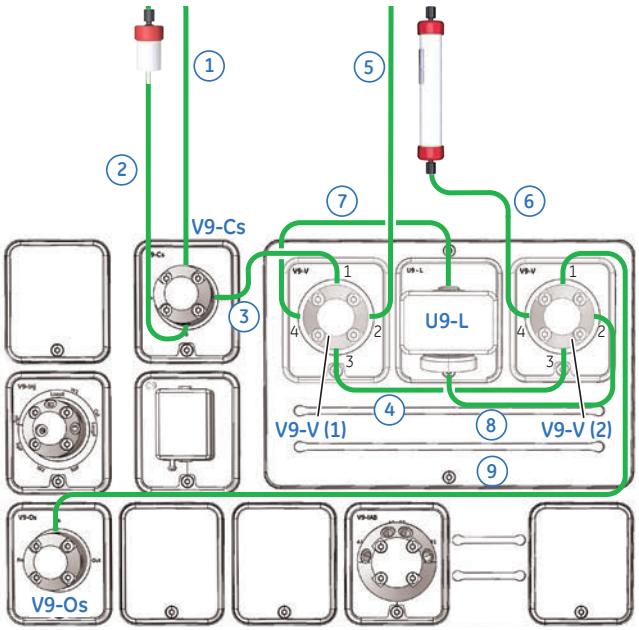
No.	From	port	To	port
1	V9-C or V9-Cs[1]	1A	Column 1	inlet
2	V9-C or V9-Cs[1]	1B	Column 1	outlet
3	V9-C	Out	V9-V(1)	1
4	V9-V(1)	3	V9-V(2)	3
5	V9-V(1)	2	Column 2	inlet
6	V9-V(2)	4	Column 2	outlet
7	V9-V(1)	4	UV monitor	inlet
8	V9-V(2)	2	UV monitor	outlet
9	V9-V(2)	1	V9-O	inlet

1 V9-Cs only applicable for ÄKTA pure as V9-C is always present in ÄKTA avant.

Note: *To distinguish between the two versatile valves, set the node ID for Versatile valve V9-V(1) to* ***20*** *and the node ID for Versatile valve V9-V(2) to* ***21****. See ÄKTA pure User Manual and ÄKTA avant User Manual for details.*

Important

Read *ÄKTA pure Operating Instructions* or *ÄKTA avant Operating Instructions* before using the instrument.



The illustration shows the flow path allowing tandem two-step purification using the UV monitor **U9-L** installed on ÄKTA pure[1].

[1] *Only applicable for ÄKTA pure since U9-M is always present in ÄKTA avant.*

Note: *Only some optional modules and tubing are included in this picture.*

Note: *Select tubing id that matches the current tubing kit used. Minimize the tubing length for optimal results.*

Note: *If the UV monitor* **U9-M** *is used instead of* **U9-L**, *position the two versatile valves as close to the UV flow cell as possible to minimize the delay volumes in the instrument.*

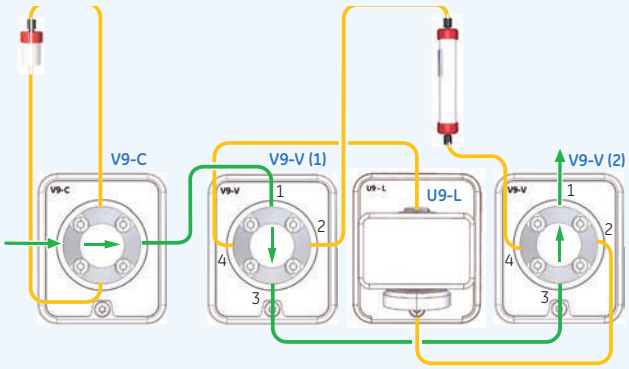
Valve positions and functionality

The two versatiles valves, UV monitor and second column constitute one unit with a flow path configuration that is dependent on the valve positions used in the versatile valves. Below are example flow path configuration shown for ÄKTA pure.

Four different flow path configurations are used:

1. Priming of the system

Both columns and UV monitor are offline.



Valve positions

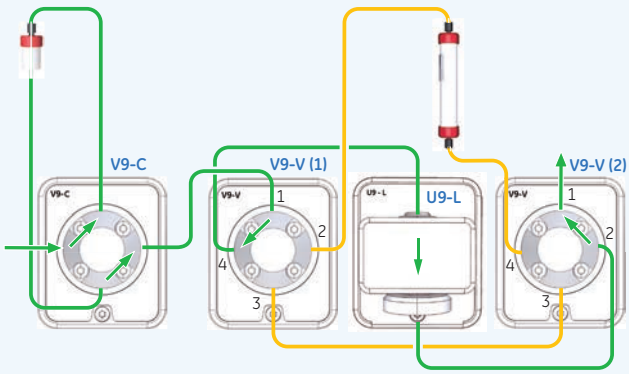
- V9-C: **By-pass**
- V9-V (1): **1** - **3**
- V9-V (2): **1** - **3**

2. Purification step one: loading and wash of column one

First column and UV monitor are inline.

Second column is offline.

UV monitor measures after the first column.



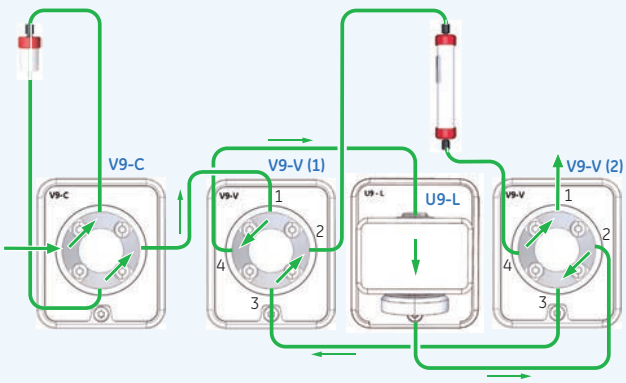
Valve positions

- V9-C: **1**
- V9-V (1): **1** - **4** & **2** - **3**
- V9-V (2): **1** - **2** & **3** - **4**

3. Purification step one: elution and loading column two

Both columns and UV monitor are inline.

UV monitor measures after the first column.



Valve positions

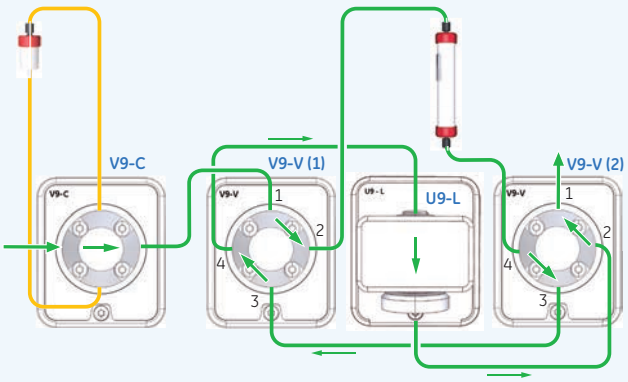
- V9-C: **1**
- V9-V (1): **1** - **4** & **2** - **3**
- V9-V (2): **1** - **4** & **2** - **3**

4. Purification step two: wash and elution of column two

Second column and UV monitor are inline.

First column is offline.

UV monitor measures after the second column.



Valve positions

- V9-C: **By-pass**
- V9-V (1): **1** - **2** & **3** - **4**
- V9-V (2): **1** - **2** & **3** - **4**

UNICORN methods

Method one

Objective

Perform the first purification step. After sample loading and wash, the eluted peak of interest is directed onto the second column.

Description

The sample is loaded onto column one and during elution and when the ***watch*** condition for peak start is fullfilled, valves turn into position ***Loading column two***.

The UV monitor is located after column one. When the peak has passed the UV monitor, the flow is directed onto the second column.

Note: *The example below allows one detected peak to be loaded onto the second column.*

Note: *The second column has to be equilibrated and ready for use prior to the method start.*

Method two

Objective

Perform the second purification step on column two, with the protein fraction loaded on column two in the first purification step, and collect fractions of the eluted peaks.

Description

The sample is eluted from column two and the column is equilibrated. The Elution and Equilibration phases are preceded by user defined phases to set the valve configuration to Wash & Elution step two position.

The UV monitor is located after the second column in order to monitor the peak elution.

How to add user defined phases to a method

Create and edit phases

The UNICORN ***Method Editor*** software is used when creating and editing phases. Follow the steps below to create a user defined phase:

- Rename a global phase
- Add new text instructions
- A user defined phase can be saved in the ***Phase Library*** under ***Global Phases*** or ***Personal Phases*** for future use.

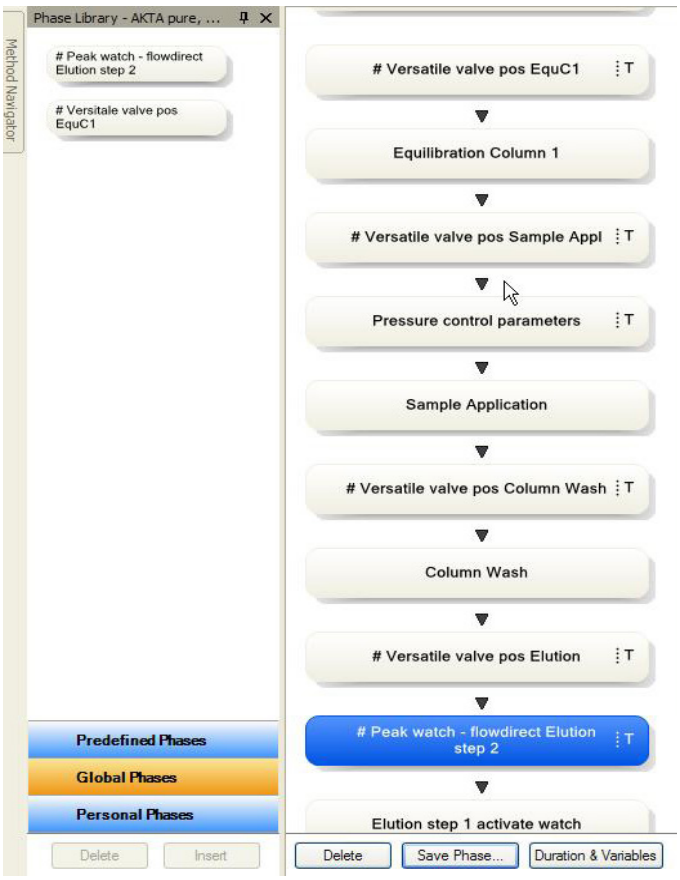
For easy identification, the modified phases used in this application were renamed starting with a ***#*** symbol.

The ***:T*** symbol is a software generated indication for a text edited phase.

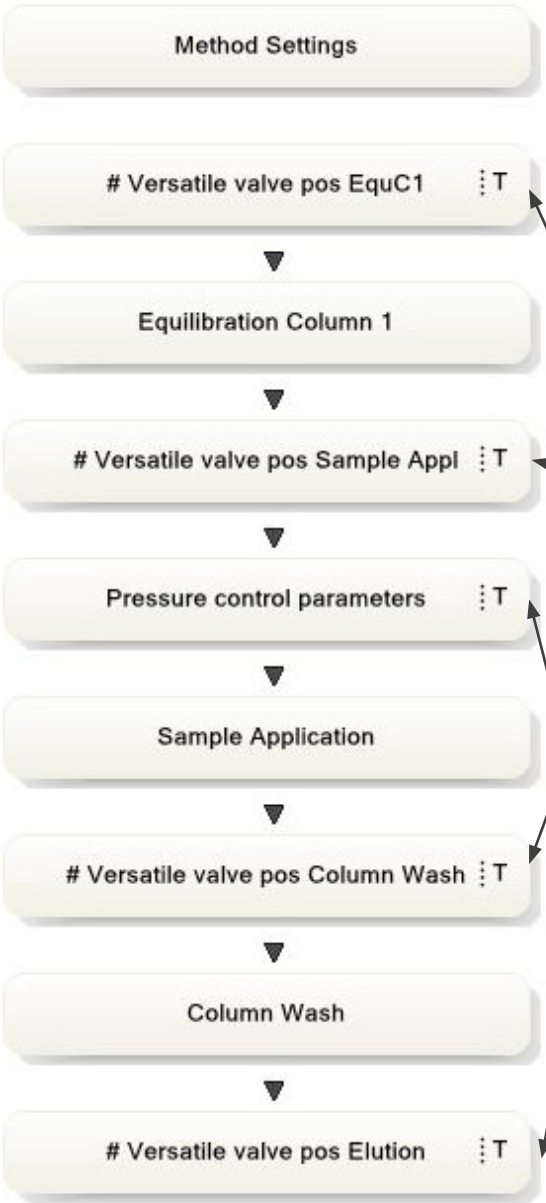
Note: *For a comprehensive guide to creating methods that can be run on ÄKTA pure and ÄKTA avant systems, refer to the UNICORN Method Manual.*

For an explanation of the used methods, see next pages.

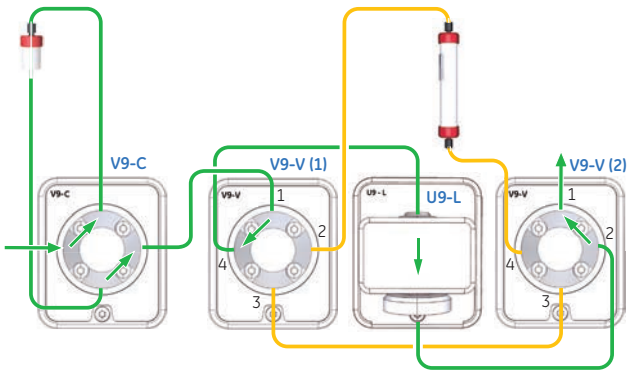
The illustration below shows an example of a UNICORN method that can be used for purification step one.

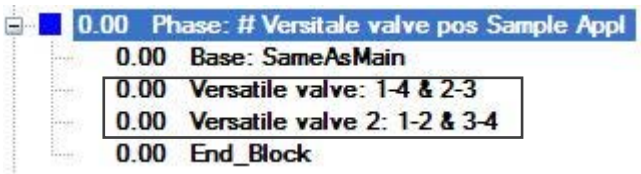


Structure and content of a method used for purification step one



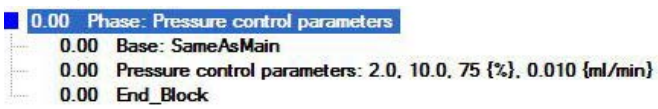
User defined phases to set the valve configuration to the Wash and Elution steps.





Optional user defined phase can be used to set new pressure control parameters to facilitate crude sample loading and minimize oscillating behaviour of the flow rate.

Tip: *Lower the value of I and P to get a slower, smoother response in flow rate adjustment. See an example below.*



In this ***Pressure control parameters*** example, ***P*** = 2.0,
I = 10.0 and target value for pressure control = 75 {%}. The values for ***P*** and ***I*** can vary greatly. The default values are ***P*** = 8.0, ***I*** = 40.0 and target value for pressure control = 90 {%}.

Structure and content of a method used for purification step one (continued)

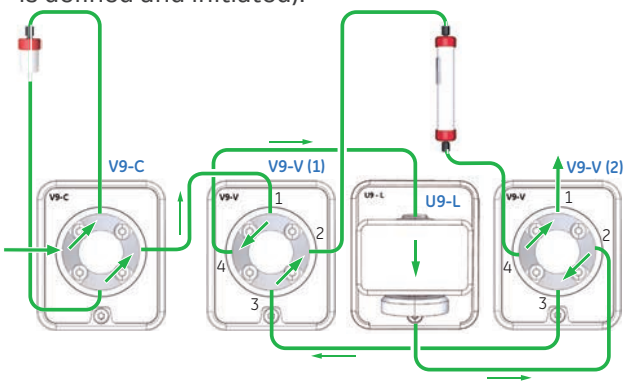
Peak watch - flowdirect Elution step 2

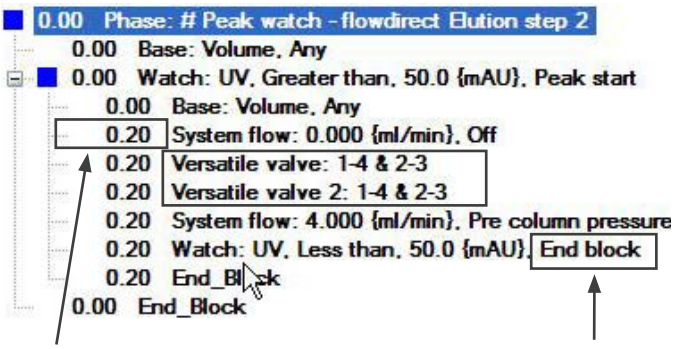
Elution step 1 activate watch

Delay peak end

User defined phase for defining:

- Watch instructions during the elution, used for peak detection.
- Valve configuration to direct/transfer detected peak to the second column (the valve configuration is defined and initiated).





Delay volume from UV monitor to the second column.

Watch instruction that detects peak end terminates the elution phase when peak end is detected.

Note: *To calculate delay volumes, the volume of each component can be found in ÄKTA pure User Manual and ÄKTA avant User Manual. The volume of tubing can be calculated using the formula:*
Volume (ml) = Length (mm) × (i.d. (mm))^2 × π /4.

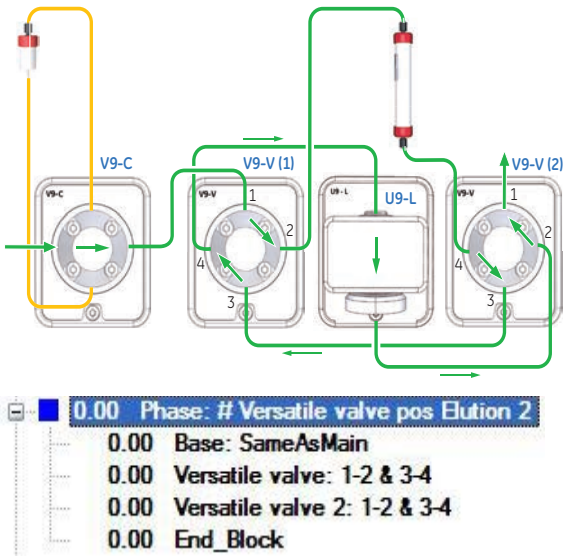
- Elution phase.
- Previously set watch commands will be active during the elution.
- The ***Watch*** instruction for peak end triggers the end of the elution phase.

Renamed ***Miscellaneous*** phase introducing a delay.

The delay is necessary because the last part of the peak has to leave the UV monitor and enter the second column before method one ends.

Structure and content of a method used for purification step two

Instructions to set valve configuration to ***Wash & Elution*** position. Illustration shows ÄKTA pure as an example.



Recommendations

Peak detection

Set ***watch*** limits so that end peak is not triggered by start peak values. For example, set ***start peak*** to greater than 100 mAU and ***end peak*** to less than 100 mAU.

Another way is to use the instruction ***Peak_start_max*** before the peak end instruction (see *UNICORN Method Manual*).

The peak volume should be larger than the delay volume between the UV monitor and the second column. The delay volume is typically 0.1 to 0.3 ml.

Column selection

Take proper care in selecting the column for the second step, especially with respect to maximum load volume but also with respect to pressure limits when both columns are in the flow path.

Good combinations are:

Purification step one	Purification step two
1 ml HiTrap™	2 × 5 ml HiTrap Desalting columns in series
1 ml HiTrap	Gel filtration/Size exclusion column, i.d. 16 mm
5 ml HiTrap	HiPrep™ Desalting
5 ml HiTrap	Gel filtration/Size exclusion column, i.d. 26 mm

Column CIP and Equilibration

- It is recommended that equilibration of column two is performed as a first method in the method queue executed prior to starting method one. This ensures that step two is ready to start with the elution step.
- CIP of column one can preferably be a dedicated method in the method queue executed as the last method.

Other options

- For loading larger sample volumes, a Sample pump or the System pump in combination with a Mixer valve, in ÄKTA pure, can be used.
- If a Sample pump is available, the addition of a sample inlet makes it possible to load the whole sample using air sensors, and also to load multiple samples.
- For full control of protein elution and simplicity, a second UV monitor can be added, allowing for simultaneous monitoring of both columns.

Download

Example methods for two-step purifications for ÄKTA pure (either equipped with Sample pump or not) and ÄKTA avant, can be downloaded from *cytiva.com/AKTA*, select ***ÄKTA pure*** or ***ÄKTA avant*** link under the heading ***Featured Products***. Then click on ***Related Documents*** tab and find the heading ***Cue Card***.

Ordering information

For ordering information on columns, valves and tubing, visit *cytiva.com/aktapure*, or *cytiva.com/aktaavant*

cytiva.com

For local office contact information, visit cytiva.com/contact.

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