Two-step purification with ÄKTA™ pure, using loop collection

Cue Card

Contents

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. The fully automated two-step purification is suitable for all column and elution combinations.

Example methods for ÄKTA pure two-step purifications can be downloaded from www.gelifesciences.com/AKTApure-software.

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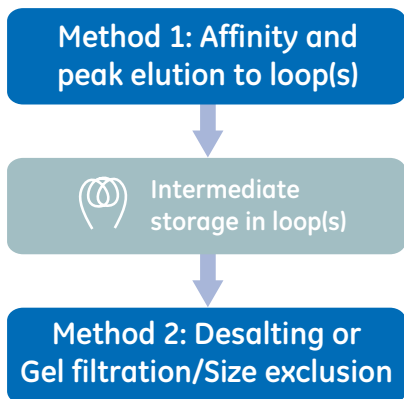
Principles

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 the column log book features can be utilized. Using a method queue allows full automation.

Method queue outline

Method 1: Affinity and peak elution to loop(s)

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Intermediate storage in loop(s)

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Method 2: Desalting or Gel filtration/Size exclusion

Method one

The user defined phase in method one defines all functionality for peak detection and loop collection of the eluted peak.

Up to 5 loops can be connected to the Loop valve **V9-L**.

Method two

The collected peak from method one is loaded onto column two. If the same volume that was collected in the loop shall be injected, a text instruction is added to the UNICORN sample loading phase in a desalting or gel filtration/size exclusion method.

Setup for multi-step purification

System configuration

Several different ÄKTA pure configurations can be used. ÄKTA pure 25 is used in the following example. To enable multistep functionality, a Loop valve **V9-L**, a Column valve **V9-C** and a Mixer valve **V9-M** or a Versatile valve **V9-V** will be needed.

Sample loading method	Modules needed
System pump	**V9-L**, **V9-C**, **V9-M**
Sample pump	**V9-L**, **V9-V**, **V9-C**, Sample pump **P9-S**

Flow path connections, when the Sample pump is used:

From	port	To	port
Pump P9-S	-	V9-V	3
V9-O or V9-Os	Out	V9-V	4
V9-V*	1	V9-Inj	SaP
V9-Inj	LoopF	V9-L	F
V9-L	E	V9-Inj	LoopE

** V9-V port **2** is not used.*

Note: *Connect a suitable loop to the Loop valve **V9-L** (see Recommendations section, Loop selection).*

	Important
	Read *ÄKTA pure Operating Instructions* before using the instrument.

Flow path connections, when the System pump is used.

No.	From	port	To	port
1	V9-O or V9-Os	Out	V9-M	Re-inj
2	V9-M	Out	V9-Inj	SaP
3	V9-Inj	LoopF	V9-L	F
4	V9-L	E	V9-Inj	LoopE
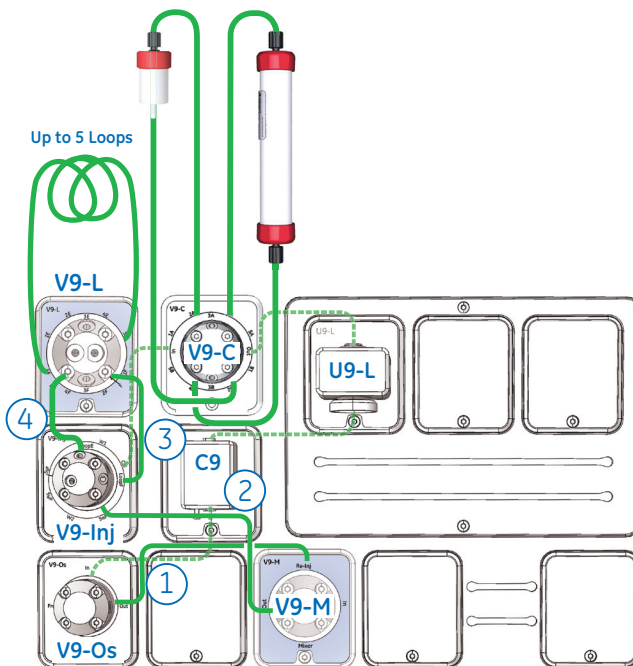
The illustration shows the two additional modules (blue) and the needed connections (green) allowing two-step purification via re-injection (System pump configuration). Standard flow path shown with dash lines.

UNICORN methods

Method one

Objective

Execute purification step one by separating the sample using peak fractionation and collecting the eluted peak from column one in a loop.

Description

During elution, when the **watch** condition for peak start is fulfilled and the set delay volume has passed, valves turn into position and the peak is directed to the loop for collection. The volume of the peak is *counted* during the collection in the loop.

Method two

Objective

Execute purification step two by loading the collected peak from method one onto a second column, wash the column and elute the sample.

Description

Add functionality to the method so that the sample injection uses the correct loop and injects the volume of the peak collected during step one.

Loop collection and injection

The volume of collected peak is counted in step one, using the **start/stop volume count** function. By inserting the instruction **hold counted volume**, the injection volume of step two can be equal to the collected peak volume. The instruction is added at the end of the **sample load** phase.

Due to the laminar flow, a larger volume than the one collected should be used during sample loading on the second column to empty the loop. Ideally, a volume that is 1.3 times larger than the collected peak volume should be used. Besides the laminar flow compensation, a compensation volume equal to the volume of the flow path from loop valve to column valve should also be added. These two volumes constitute the *chase** volume.

The instruction **Empty loop with** is used to set preferred chase volume (laminar flow compensation volume + flow path delay volume).

Note: *The desalting and size exclusion columns have limitations in the sample volumes that can be added*.

* A chase volume is the volume of buffer that is used to push (i.e. chase) substances (sample, peak, etc.) from one location in the flow path to another.
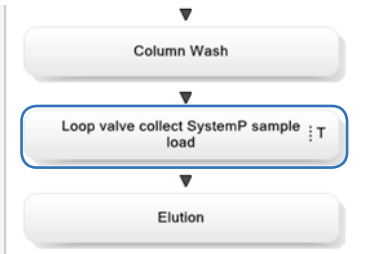
How to add user defined phases to a method

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, modified phases can be 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 an ÄKTA pure system, refer to the UNICORN 6 Method Manual.*

Method one: *Loop valve collect* phase

• The flow path for peak collection will be washed prior to activating the watch functions for peak collection.

• During elution, the watch functions will be active and, if fulfilled, will direct the peak to the loop. Details are presented in the following pages.

• After peak collection the flow path used for peak collection will be washed.

The illustration shows an example of a UNICORN method that can be used for purification step one (*Loop valve collect* phase).
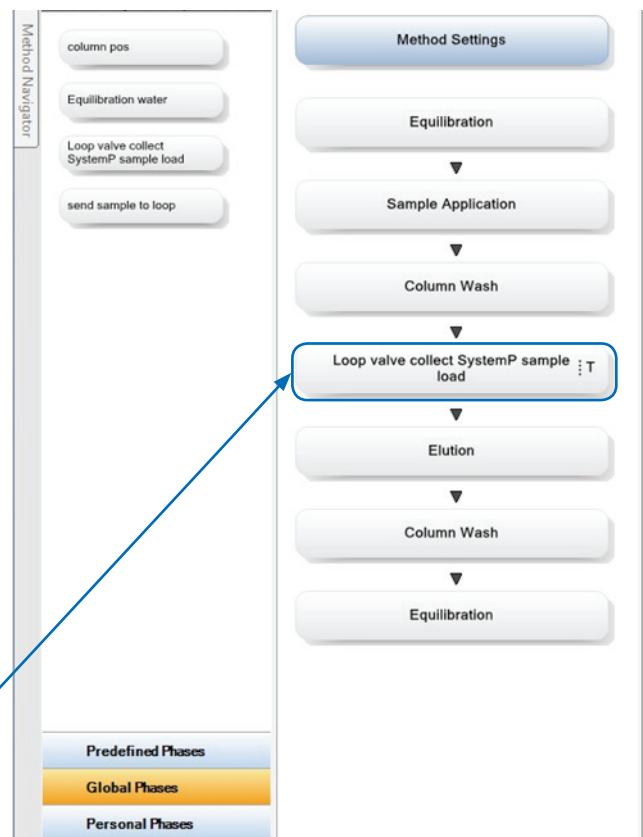
Description of the user defined phase in method one

User defined phase: *Loop valve collect / Watch* instructions

Note: The phase in this example has been designed for sample loading using the System pump and for collecting one peak. Multiple peaks can be collected using the same principle if additional watch commands are added.

Volume from UV monitor to Outlet valve. In this example 0.20 ml.

Note: The volume of each component is found in ÄKTA pure System Handbook. The volume of tubing can be calculated using the formula: Volume (ml) = Length (mm) × (i.d. (mm))² × π /4.

Position on Outlet valve where the tubing from Mixer valve **V9-M** port **Re-Inj** is connected.

*Note: If a Sample pump is used to apply the sample, the instruction where the flow is directed to V9-V (**Outlet valve: Outlet 3**) needs to be followed by an instruction that sets V9-V to position **1-4 & 2-3**.*

Position of the loop where the peak will be collected.

It uses the same variable for the flow rate as in *Method Settings*.

Volume from UV monitor to Loop valve. In this example 0.54 ml.

Block: *Loop collect flowpath wash*

Set *Base* to *Volume, Any*.

*Note: If the **Base** is connected to column volume and used flow rate for the wash is higher than the column's max flow rate, a warning will be issued.*

Position on Outlet valve where the tubing from Mixer valve **V9-M** port **Re-inj** is connected.

Flow rate used during loop collection and flow path wash.

It uses the same column position variable as in the *Method Settings*.

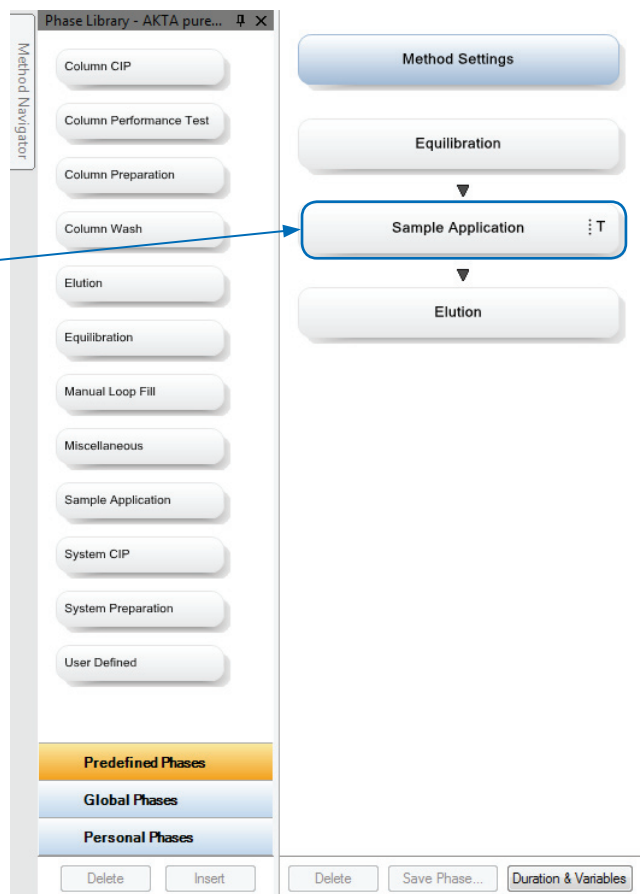It uses the same variable for the flow rate as in *Method Settings*.

*Note: The use of the same variable allows the changes in **Method Settings** to be reflected in the **Loop collect flow path wash** block. For a detailed description of variables, see UNICORN 6 Method Manual.*

Edit *Sample Application* phase in method two

Phase: *Sample Application*

The *Sample Application* phase can be edited to utilize the **Hold counted volume** UNICORN instruction.

*Note: When performing text editing of the **Sample Application** phase, the **Phase Properties** pane will be replaced with a list of phase variables.*

Block *Inject*

Additional instruction to **Sample Application** phase if injection volume should equal collected loop volume.

It is possible to set the maximum volume to be used. In this example 5.00 ml.

Chase volume (laminar flow compensation volume + flow path delay volume). In this example 1.00 ml.

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 6 Method Manual*).

The peak volume should be larger than the delay volume between the UV monitor and the Outlet valve. The delay volume is typically 0.2 to 0.5 ml.

Column selection

Take proper care in selecting the column for the second step, especially with respect to maximum load volume.

Recommended 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

Loop selection

Use at least 1.3 times the expected peak volume to collect. Recommended to use Loop holder 29-0113-50.

Column CIP and Equilibration

To minimize the time the eluted peak from step one spends in the loop before being re-injected during step two, you can create dedicated methods for cleaning and equilibration procedures.

- CIP and re-equilibration of column one can be methods executed after the method running step two of the purification.
- Equilibration of column two can be a method executed prior to starting method one.

Download

Example methods for ÄKTA pure (either equipped with Sample pump or not) can be downloaded from *www.gelifesciences.com/AKTApure-software*

Ordering information

For ordering information on columns, valves and tubing visit *www.gelifesciences.com/AKTApure*.