cytiva

How to set-up a two-step purification protocol for ÄKTA pure 25 system

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Introduction

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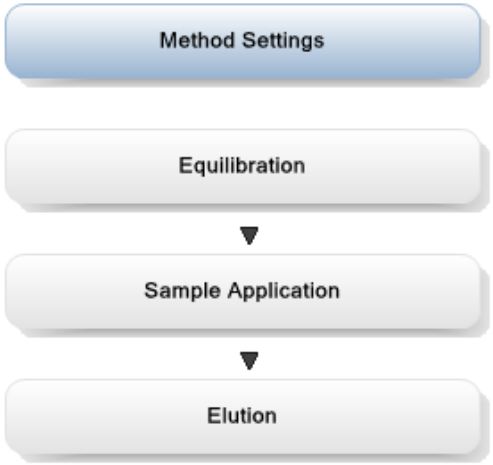
This instruction describes automated two-step protocols supporting four different system setups

1. Capture

Automatic transfer of target protein (peak) and start of second method

2. Polishing



Prerequisites

- Instrument configuration version ÄKTA™ pure 25 (1.10.0.6)
- Peak Collect tubing kit to direct peak to subsequent steps, product code 29314678
- **Method Queue** functionality to run capture and polishing steps without manual interactions during run

Conceptual overview

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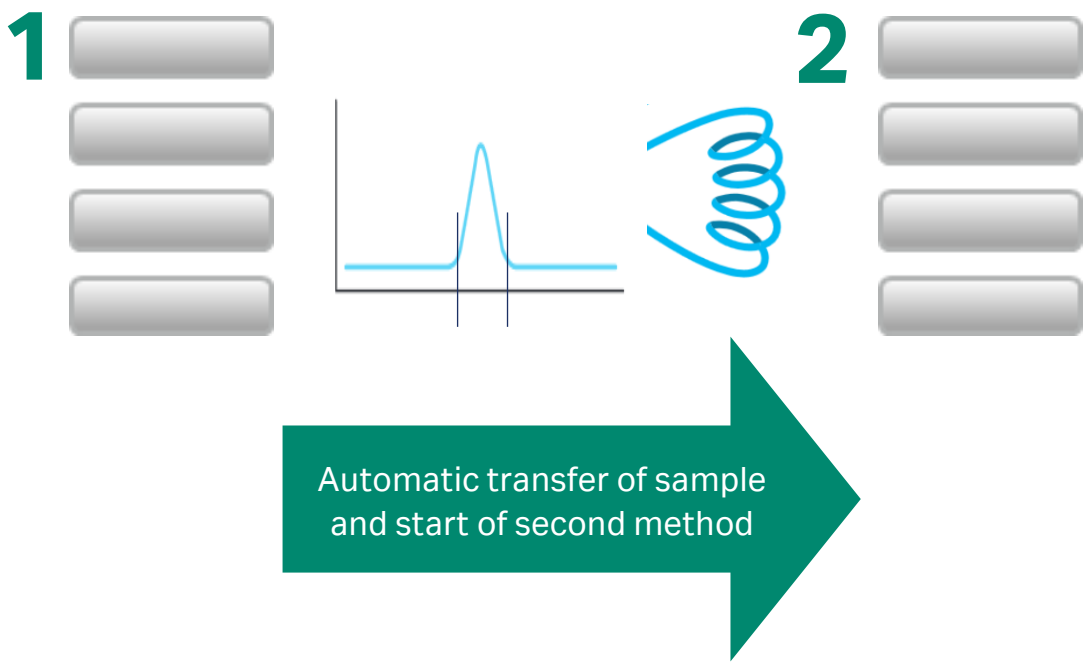
Method setup for an automated two-step purification

Include first and second method in a method queue



1. Set up your methods for the two steps as individual methods, using the predefined protocols:
   - Capture step*

     Define how to load sample (from loop or direct injection)

     Use the **Peak to loop** option in **Elution** phase to direct the eluted peak from capture step to the loop

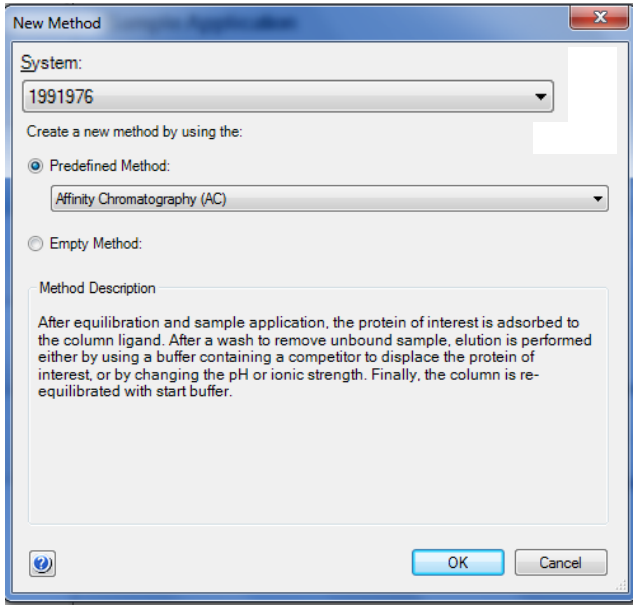
     Note: Do not use Superloop™. The loop wash included in the method can cause overpressure and damage the Superloop.
   - Polishing step

     Collect fractions in a fraction collector or in outlet valve V9-O
2. Create a method queue and start the run in **System Control.**

* Any binding chromatographic technique can be used, however, sample condition compatibility and volume limits for subsequent size exclusion chromatography (SEC) need to be considered.

How to set up the method for a capture step

Select predefined method to be used



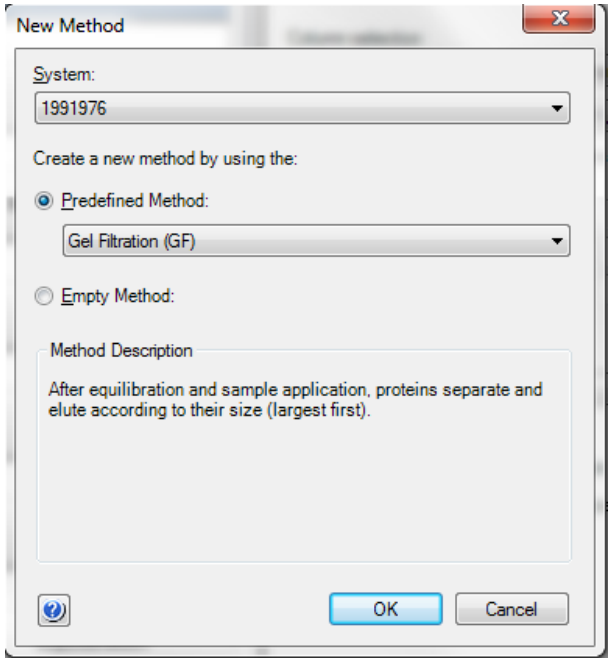
The Method Outline is displayed

Adjust phase properties

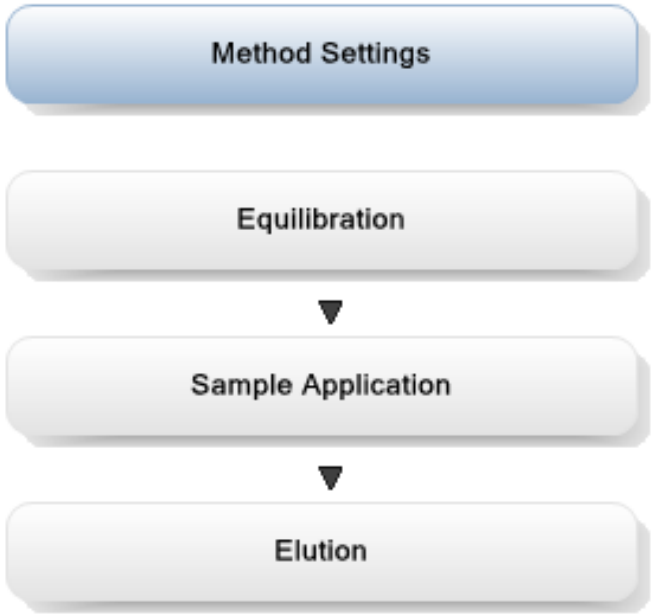
- Edit **Phase Properties** as appropriate
- Select sample injection technique according to available system components
- Select the **Peak to loop** option in the **Elution** phase

How to set up the method for a polishing step

Select predefined method to be used



The Method Outline is displayed



Adjust Phase Properties

Edit **Phase Properties** as appropriate.

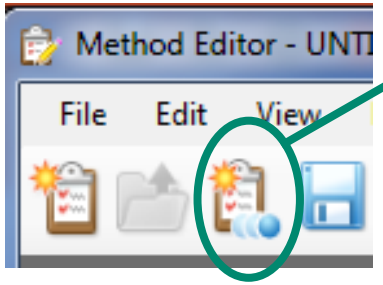
Important: Make sure to select to inject sample from loop and define the volume used to empty the loop with*.

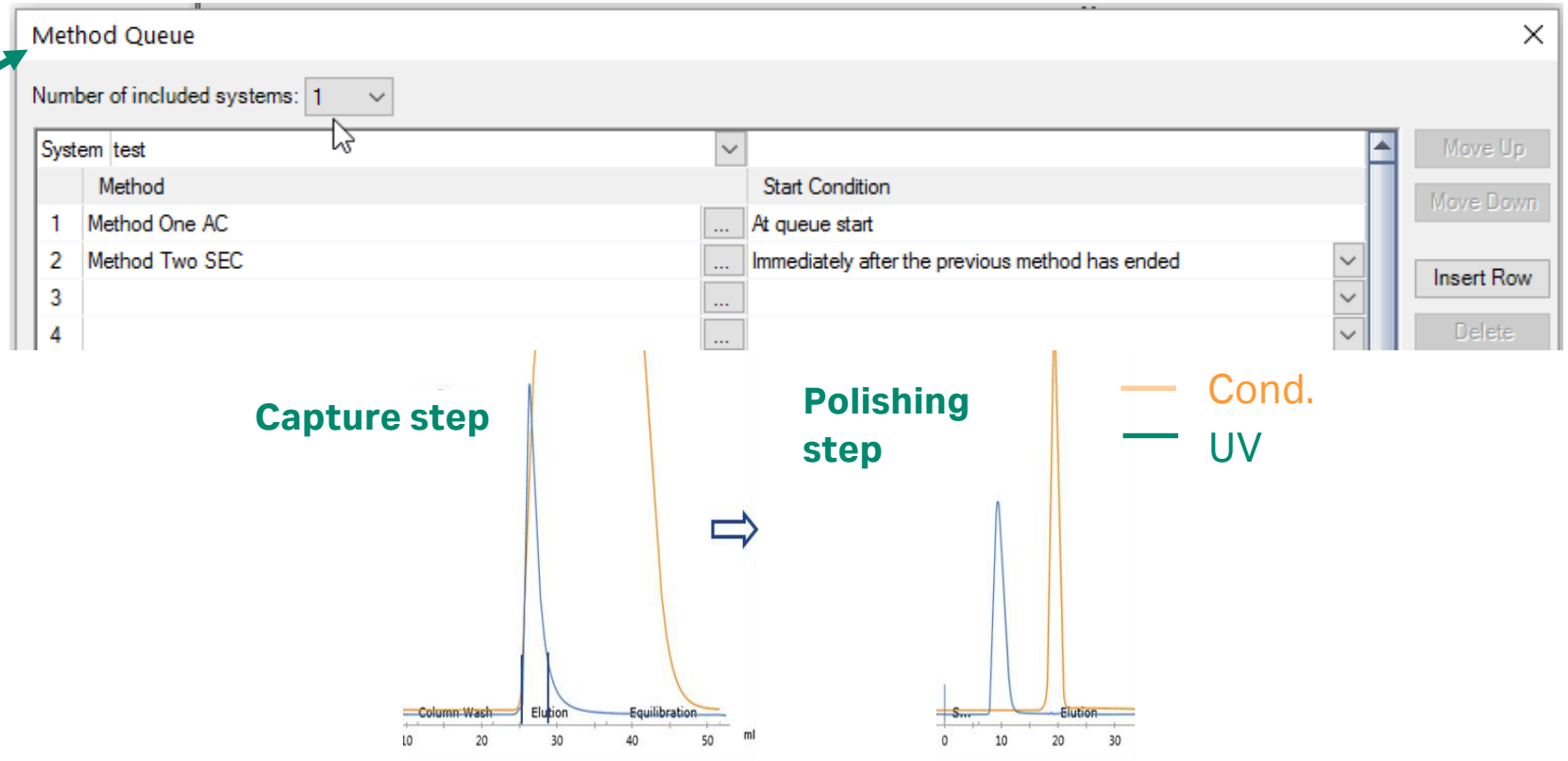
Note: Do not use Superloop™. The loop wash included in the method can cause overpressure and damage the Superloop.

* To improve recovery, use an excess volume of at least three times the loop volume to empty the loop. If SEC is used, make sure that the allowed load volume is not exceeded.

Method setup: create **Method Queue** — include the capture and polishing step in **Method Queue**

Method Queue is set in the Method Editor in UNICORN™ control software



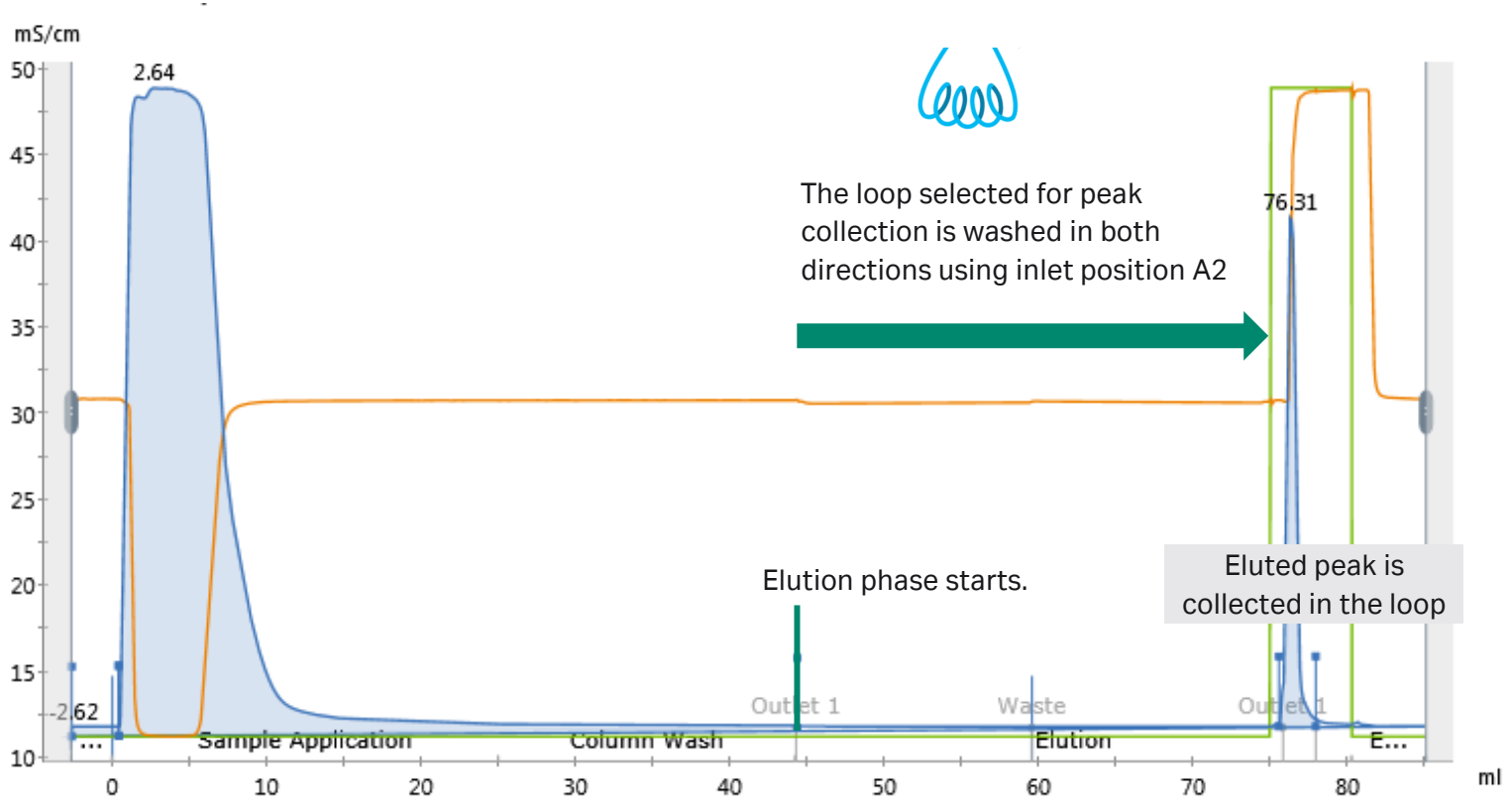


What happens during the capture step?

1. Sample applied using selected injection technique.
2. Unbound sample washed out and by default collected in the outlet valve waste position.
3. Selected loop for peak collection washed.

   Note: Connect inlet A2 to buffer A to ensure correct conditions in polishing step.
4. When peak is detected, outlet valve switches to direct flow to loop. At peak end it switches back to previous position.

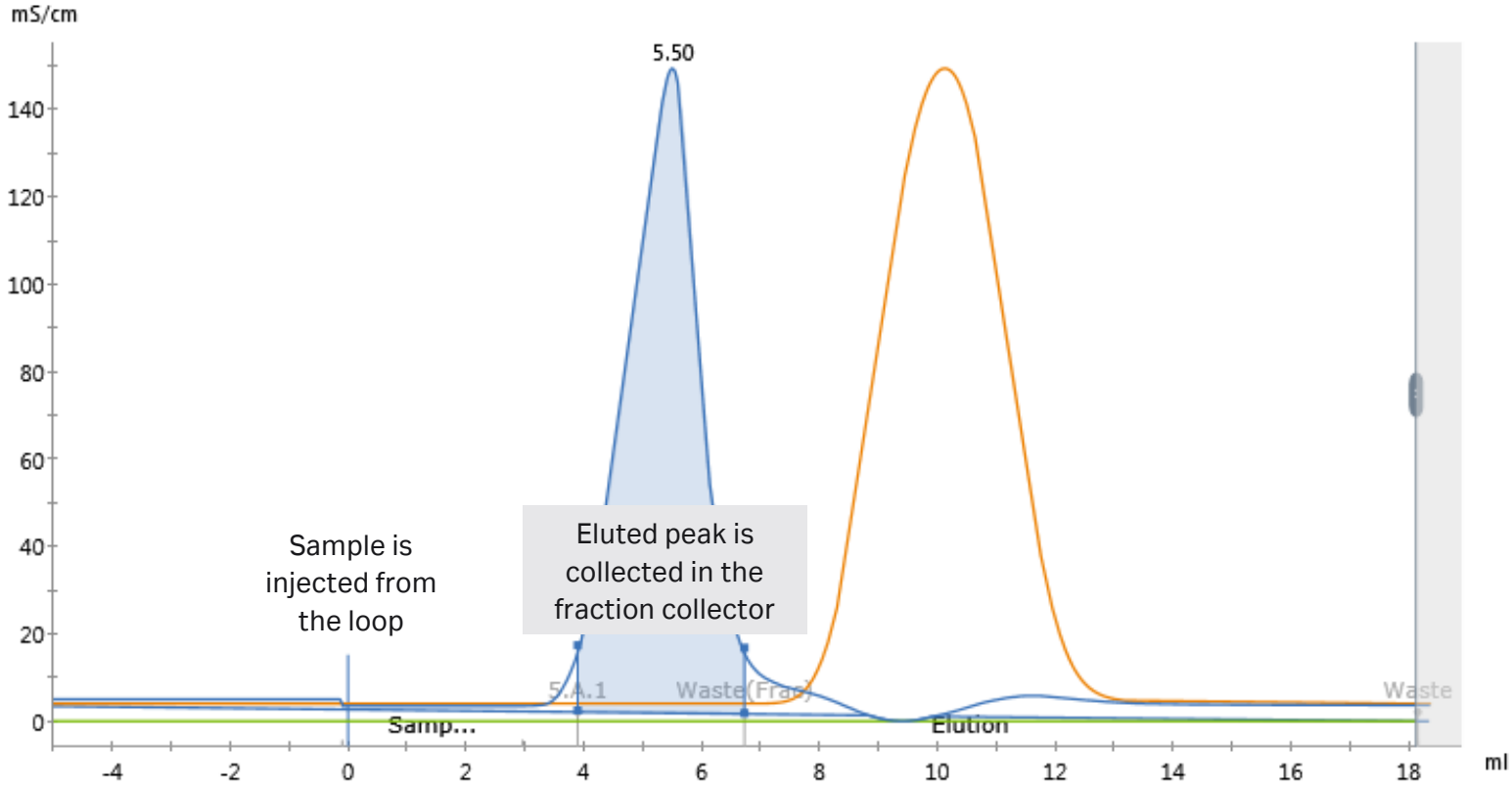
   Note: Method will compensate for delay volume between outlet and loop based on volume in peak collect tubing.



What happens during the polishing step?

1. Sample is applied from loop used to collect peak in capture step.
2. Eluted peak from polishing step is collected in the fraction collector.

Note: Remember to select use of fraction collector in the elution phase at method creation.



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Supported system configurations

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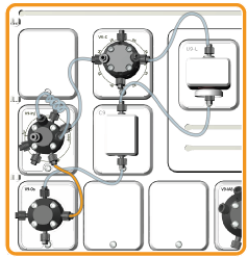
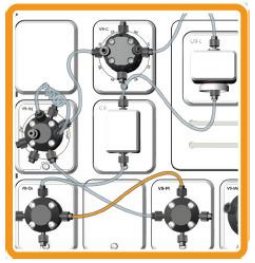
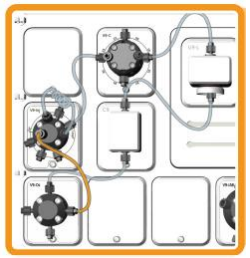
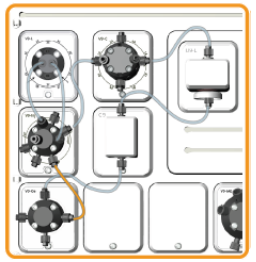
Description of supported system set-ups for sample loading and peak collection during capture step

	Basic set-up	Set-up with mixer valve	Set-up with sample pump	Set-up with loop valve
				
Tubing	Peak collect tubing between Injection valve position SaP and Outlet valve position Out (V9-Os) OR Out 2 (V9-O)	Peak collect tubing between Mixer valve position ReInj and Outlet valve position Out (V9-Os) OR Out 2 (V9-O)	Peak collect tubing between Injection valve position SyR and Outlet valve position Out (V9-Os) OR Out 2 (V9-O)	Peak collect tubing between Injection valve position SaP and Outlet valve position Out (V9-Os) OR Out 2 (V9-O)
Sample loading	From loop on Injection valve	System pump	Sample pump	From loop valve connected to Injection valve
Intermediate peak	Intermediate peak collected in a Sample loop attached to the Injection valve	Intermediate peak collected in a Sample loop attached to the Injection valve	Intermediate peak collected in a Sample loop attached to the Injection valve	Intermediate peak collected in Sample loop attached to Loop valve

Benefits for these four different ÄKTA pure system configurations

Basic set-up

Benefits:

+ **No further automation components needed**
+ Syringe port on Injection valve free (no replumbing)

Limitations:

- Uses same sample loop for first sample application and peak collection
- Limited sample volume

Overview of basic ÄKTA™ pure configuration

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Mixer valve configuration

Benefits:

+ **Unlimited sample volume**
+ Syringe port on Injection valve free (no replumbing)

Limitations:

- Not optimal for small sample volumes

Overview of ÄKTA pure configuration with mixer...

Sample pump configuration

Benefits:

+ Unlimited sample volume
+ **Loading sample through sample pump** (to minimize cross contamination)

Limitations:

- Syringe port on Injection valve used for intermediate peak collection (replumbing required)

Overview of ÄKTA pure configuration with sample pump

Loop valve configuration

Benefits:

+ **Most versatile option** (can be combined with sample pump or mixer valve)
+ Syringe port on Injection valve free (no replumbing)
+ Minimized risk of cross-contamination in multistep

Limitations:

- Not optimal for small peaks due to large delay volumes for peak collect and load to next step

Overview of ÄKTA pure configuration with loop ...

Overview of the basic ÄKTA pure configuration

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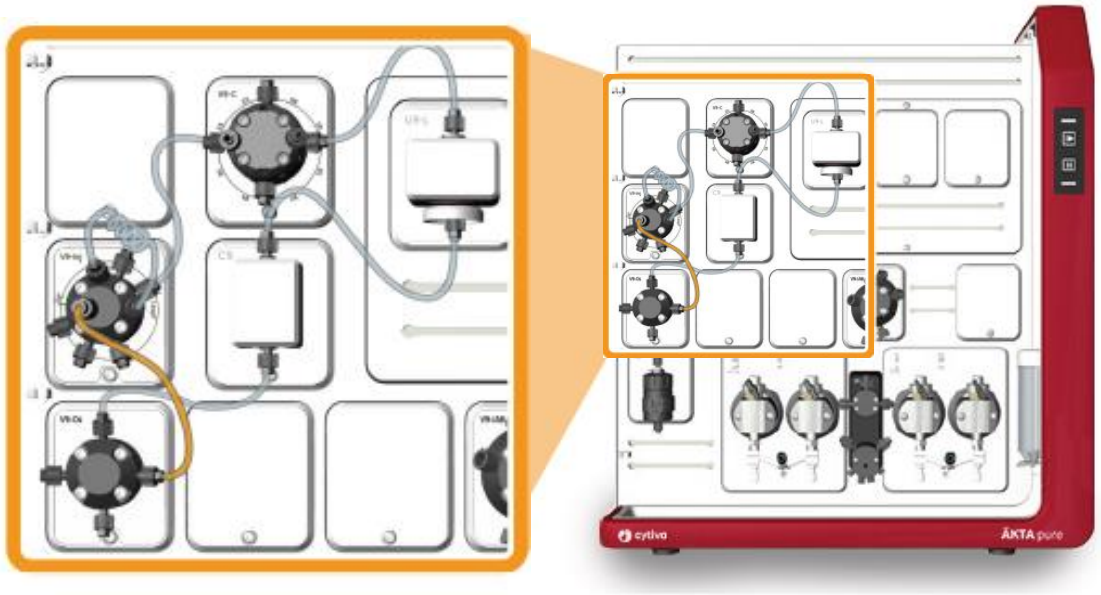
Two-step purification with intermediate peak collection in loop

ÄKTA™ pure basic configuration set-up



The intermediate peak is directed to the sample loop on the Injection valve before going on to the second column.

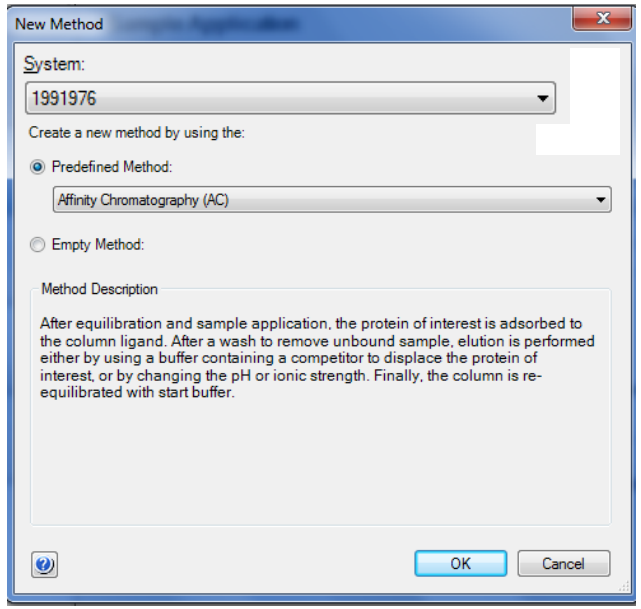
Required components

- Inlet valve V9-IA or V9-IAB
- Injection valve V9-Inj
- Tubing loop for loading sample in first step and collection of intermediate peak
- Column valve for 5 columns V9-C
- UV monitor U9-M or U9-L
- Outlet valve V9-O or V9-Os
- Fraction collector F9-C or F9-R
- Peak collect tubing 25

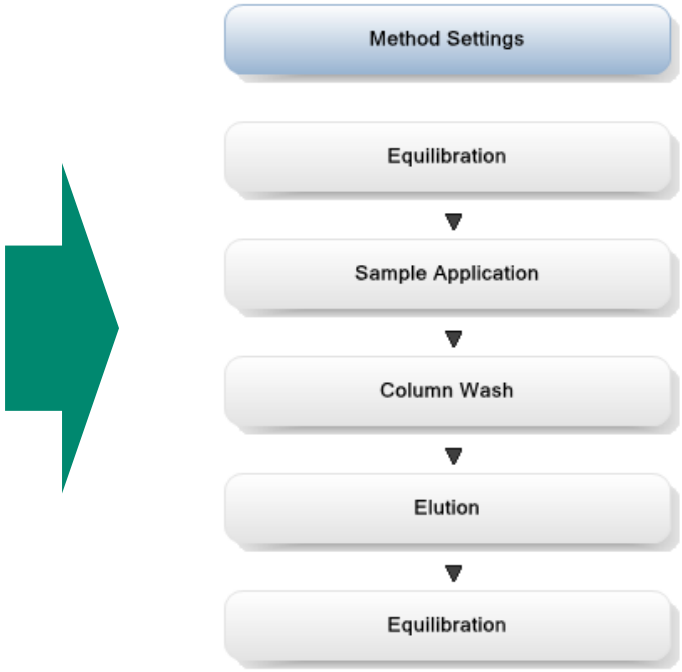
Connect the Peak collect tubing from Out/Out 2 to SaP port on the Injection valve.

How to set up the method for a capture step in the ÄKTA pure basic configuration set-up

Select predefined method to be used



The Method Outline is displayed



Adjust Phase Properties

Edit **Phase Properties** as appropriate.

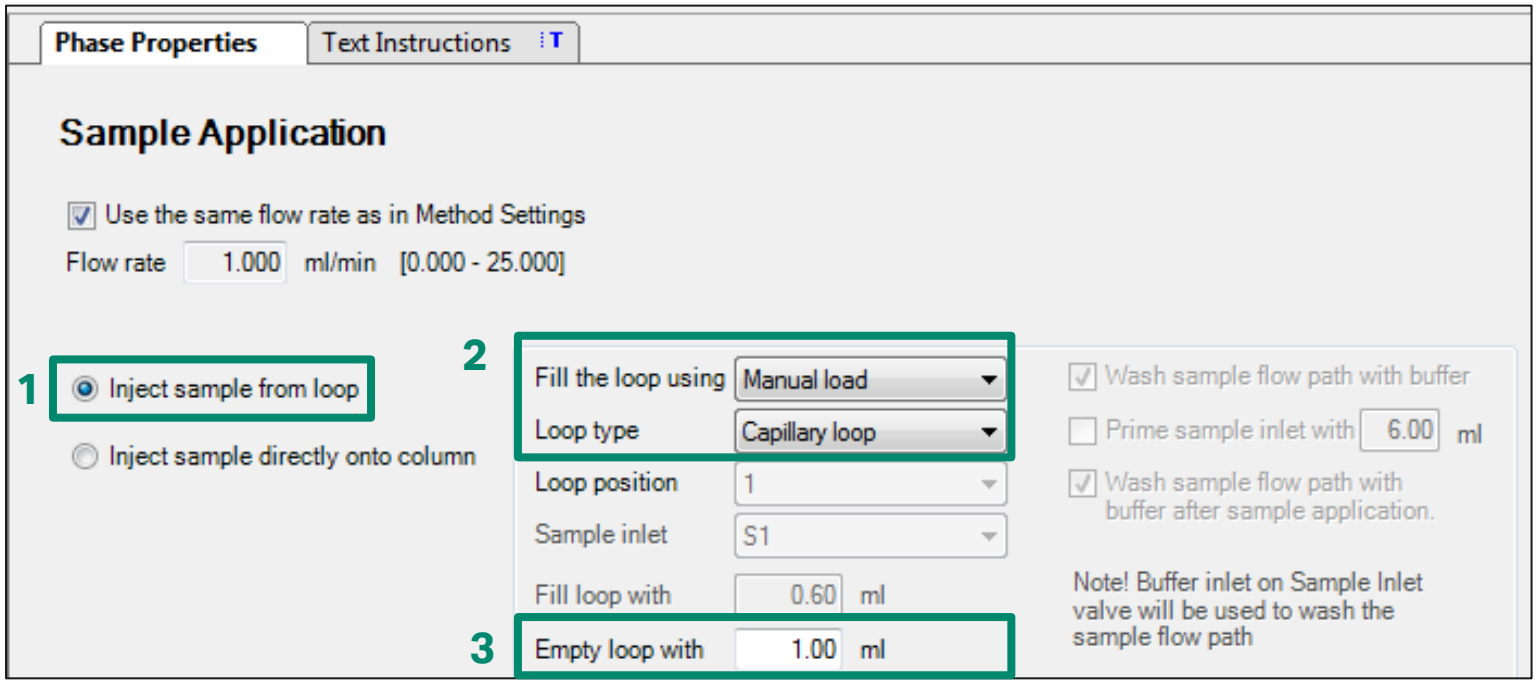
Important: Make sure to select to inject sample from loop and define the volume used to empty the loop with*.

Important: Use the **peak to loop** option in **Elution** phase.

Note: Do not use Superloop™. The loop wash included in the method can cause overpressure and damage the Superloop.

* To improve recovery, use excess volume of at least three times the loop volume to empty loop. If SEC is used, ensure that allowed load volume is not exceeded.

How to set up method for a capture step — sample application in the ÄKTA pure basic configuration set-up

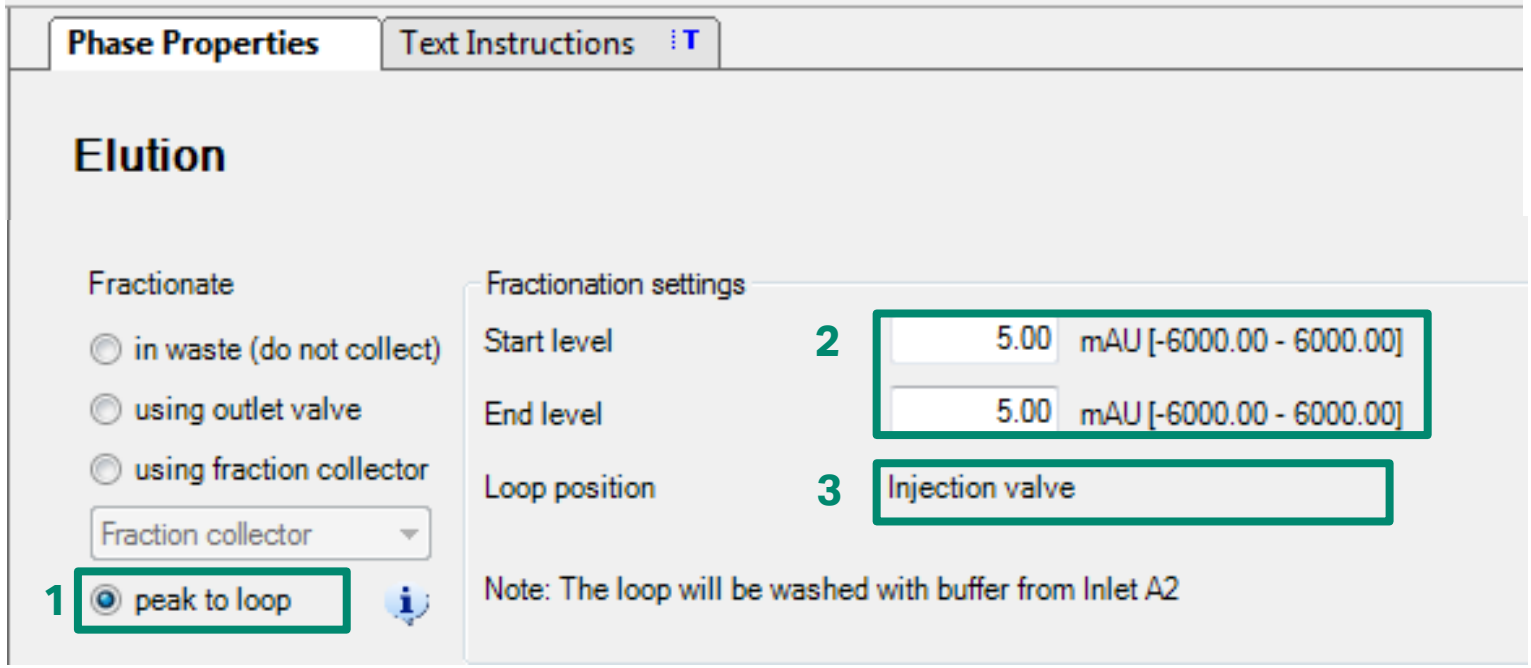


Adjust **Phase Properties** for **Sample Application**

1. Select to inject the sample from loop.
2. Select to fill the loop manually and define type as Capillary loop.
3. Define the volume used to empty loop with*.

* To improve recovery, use an excess volume of at least three times the loop volume to empty the loop. If SEC is used, make sure that the allowed load volume is not exceeded.

How to setup the method for a capture step — elution in the ÄKTA pure configuration basic set-up



Adjust **Phase Properties** for **Elution**

1. Select to collect the peak in a loop*.
2. Define UV levels for peak start and end identification.
3. The peak will be collected in the loop on the Injection valve**.

* Remember to place Inlet A2 in buffer container as it is used for loop wash.
**Remember to connect the Peak collect tubing from “Out/Out 2” to SaP port on Injection valve.

Overview of the ÄKTA pure configuration with mixer valve

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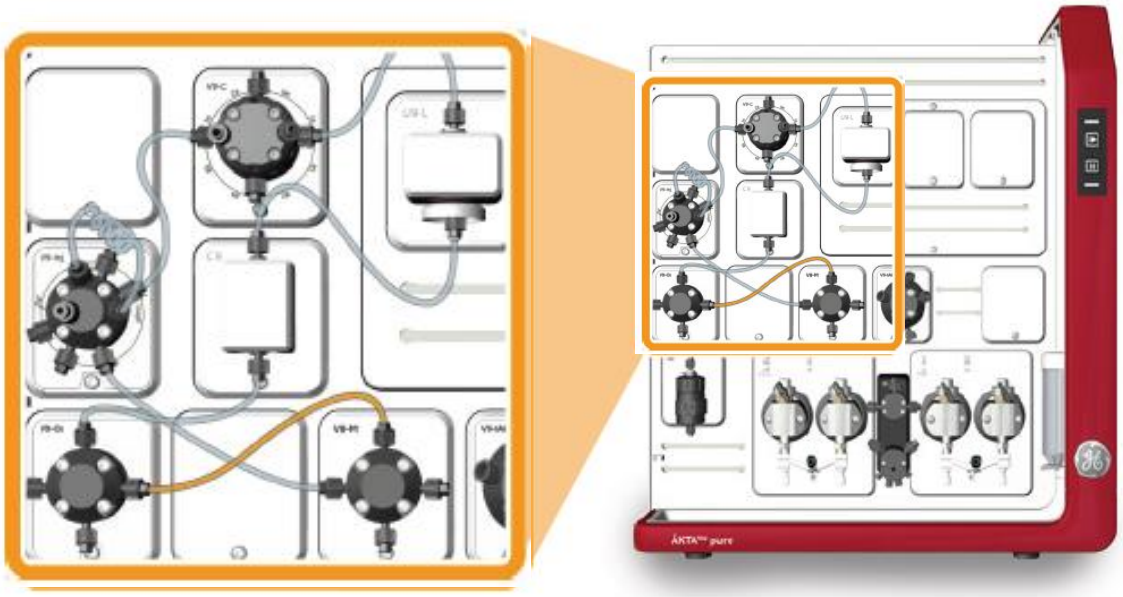
Two-step purification with intermediate peak collection in loop

ÄKTA™ pure configuration with mixer valve



The intermediate peak is directed to the sample loop on the Injection valve before going on to the second column.

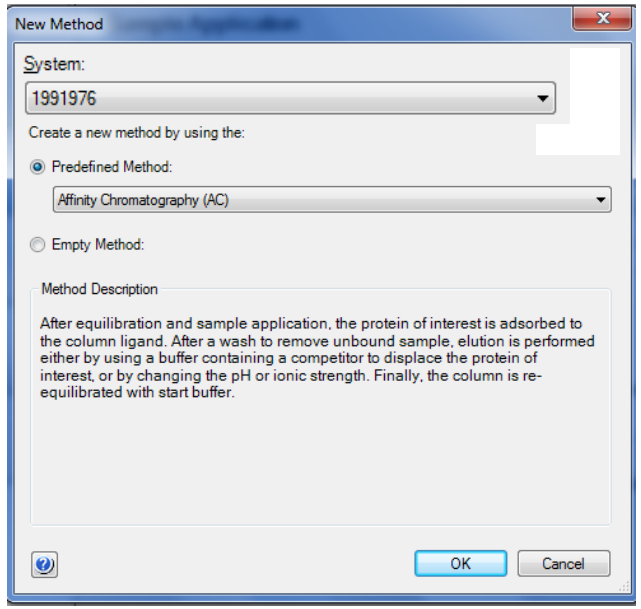
Required components

- Inlet valve V9-IA or V9-IAB
- Mixer valve V9-M
- Injection valve V9-Inj
- Tubing loop for collection of intermediate peak
- Column valve for 5 columns V9-C
- UV monitor U9-M or U9-L
- Outlet valve V9-O or V9-Os
- Fraction collector F9-C or F9-R
- Peak collect tubing 25

Connect the Peak collect tubing from Out/Out 2 to ReInj port on Mixer valve.

How to set up the method for a capture step in the ÄKTA pure configuration including a mixer valve

Select predefined method to be used



The Method Outline is displayed

Adjust Phase Properties

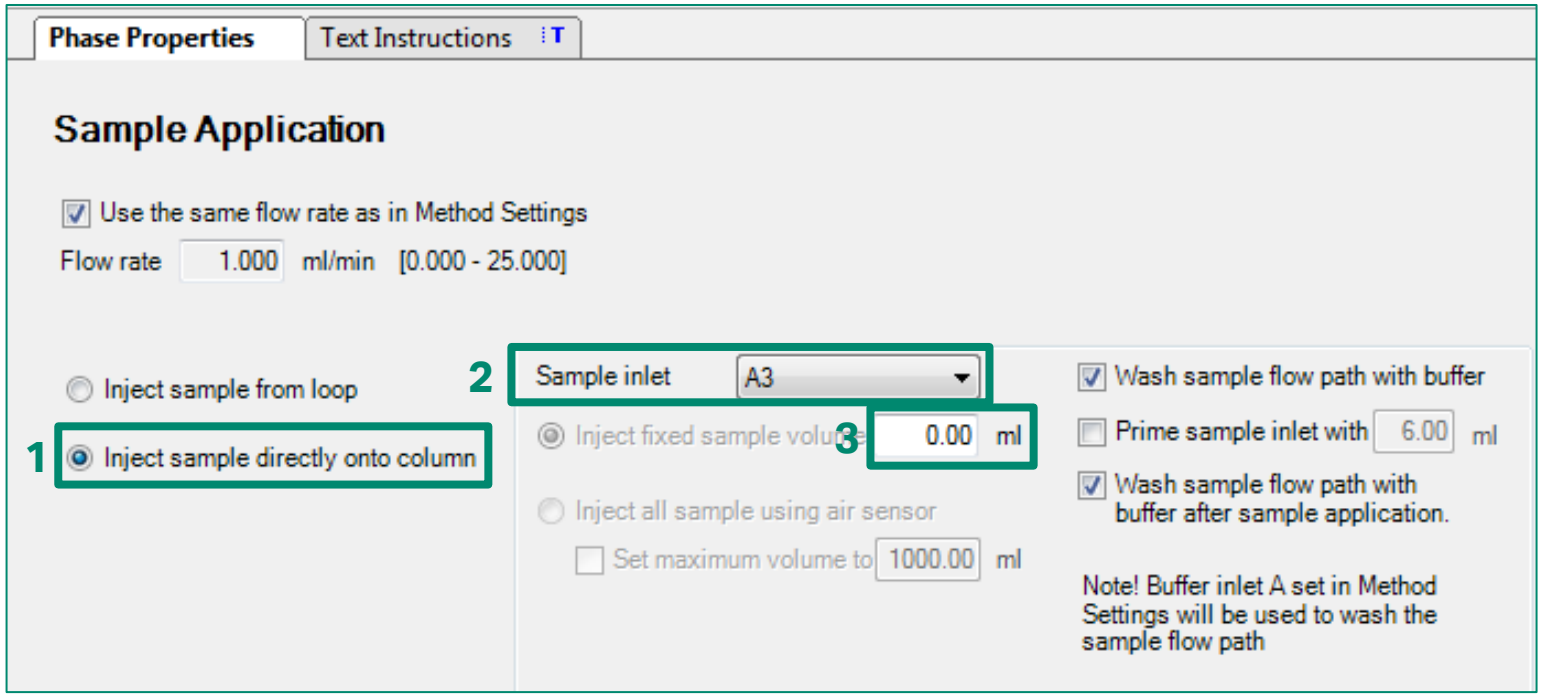
Edit the **Phase Properties** as appropriate.

Important: Make sure to select to inject sample directly onto the column in the **Sample Application** phase.

Important: Use the **peak to loop** option in **Elution** phase.

Note: Do not use Superloop™. The loop wash included in the method can cause overpressure and damage the Superloop.

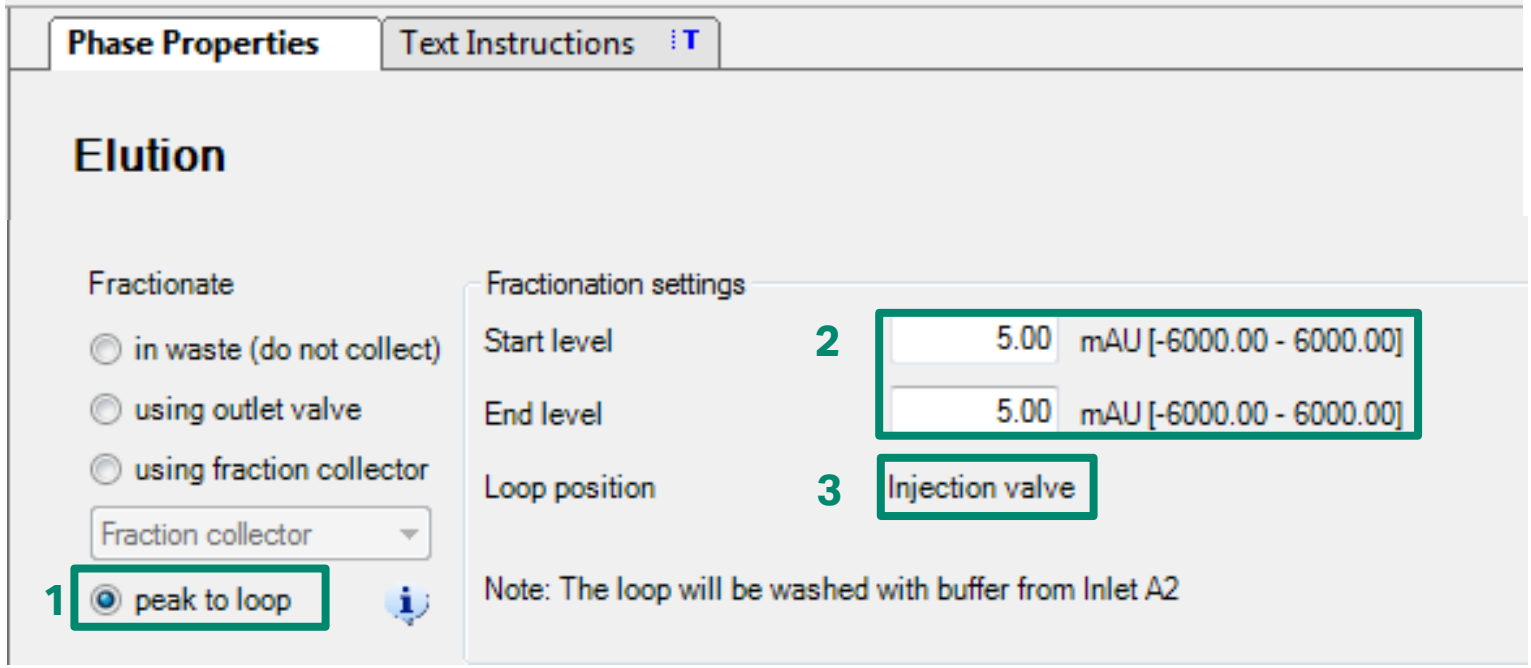
How to setup method for a capture step — sample application in the ÄKTA pure configuration including a mixer valve



Adjust **Phase properties** for **Sample Application**

1. Select to inject the sample directly onto column (the system pump will be used).
2. Define which sample inlet to use.
3. Enter the sample volume.

How to setup the method for a capture step — elution in the ÄKTA pure configuration including a mixer valve



Adjust **Phase Properties** for **Elution**

1. Select to collect the peak in a loop*.
2. Define UV levels for peak start and end identification.
3. The peak will be collected in the loop on the Injection valve**.

* Remember to place Inlet A2 in buffer container to be used for loop wash.
**Remember to connect the Peak collect tubing from Out/Out 2 to ReInj port on Mixer valve.

Overview of the ÄKTA pure configuration with sample pump

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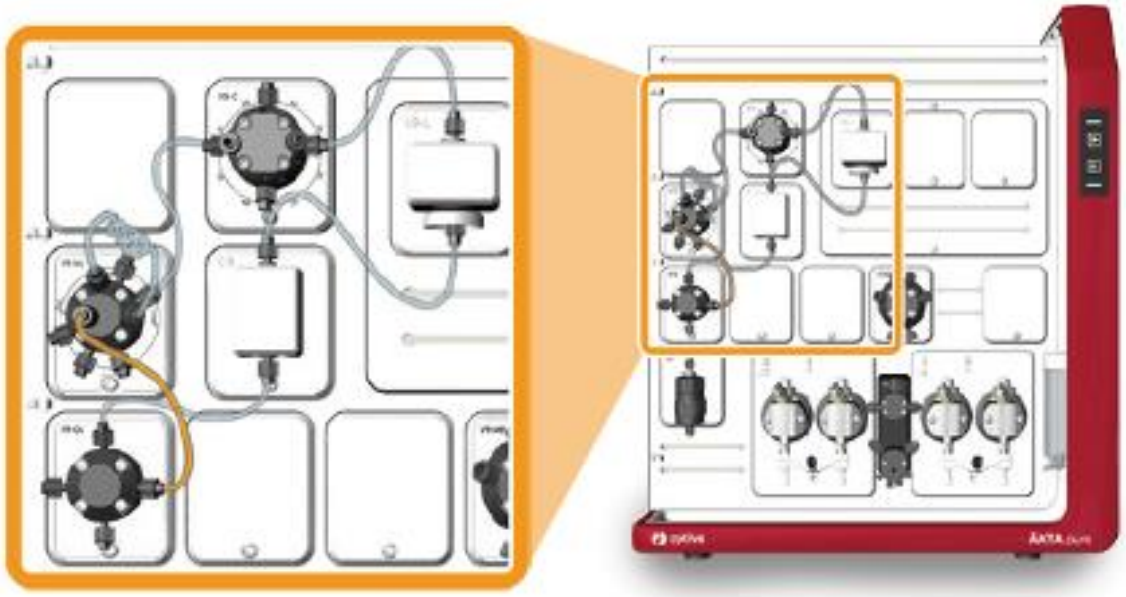
Two-step purification with intermediate peak collection in loop

ÄKTA™ pure configuration including a sample pump



The intermediate peak is directed to the sample loop on the Injection valve before going on to the second column.

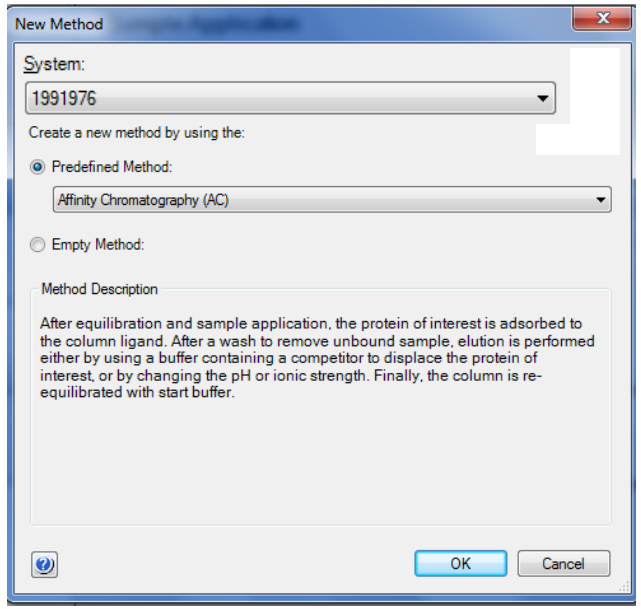
Required components

- Inlet valve V9-IA or V9-IAB
- Sample pump S9
- Injection valve V9-Inj
- Tubing loop for collection of intermediate peak
- Column valve for 5 columns V9-C
- UV monitor U9-M or U9-L
- Outlet valve V9-O or V9-Os
- Fraction collector F9-C or F9-R
- Peak collect tubing 25

Connect the Peak collect tubing from Out/Out 2 to SyR port on Injection valve.

How to setup the method for a capture step in the ÄKTA pure configuration including a sample pump

Select predefined method to be used



The Method Outline is displayed

Adjust Phase Properties

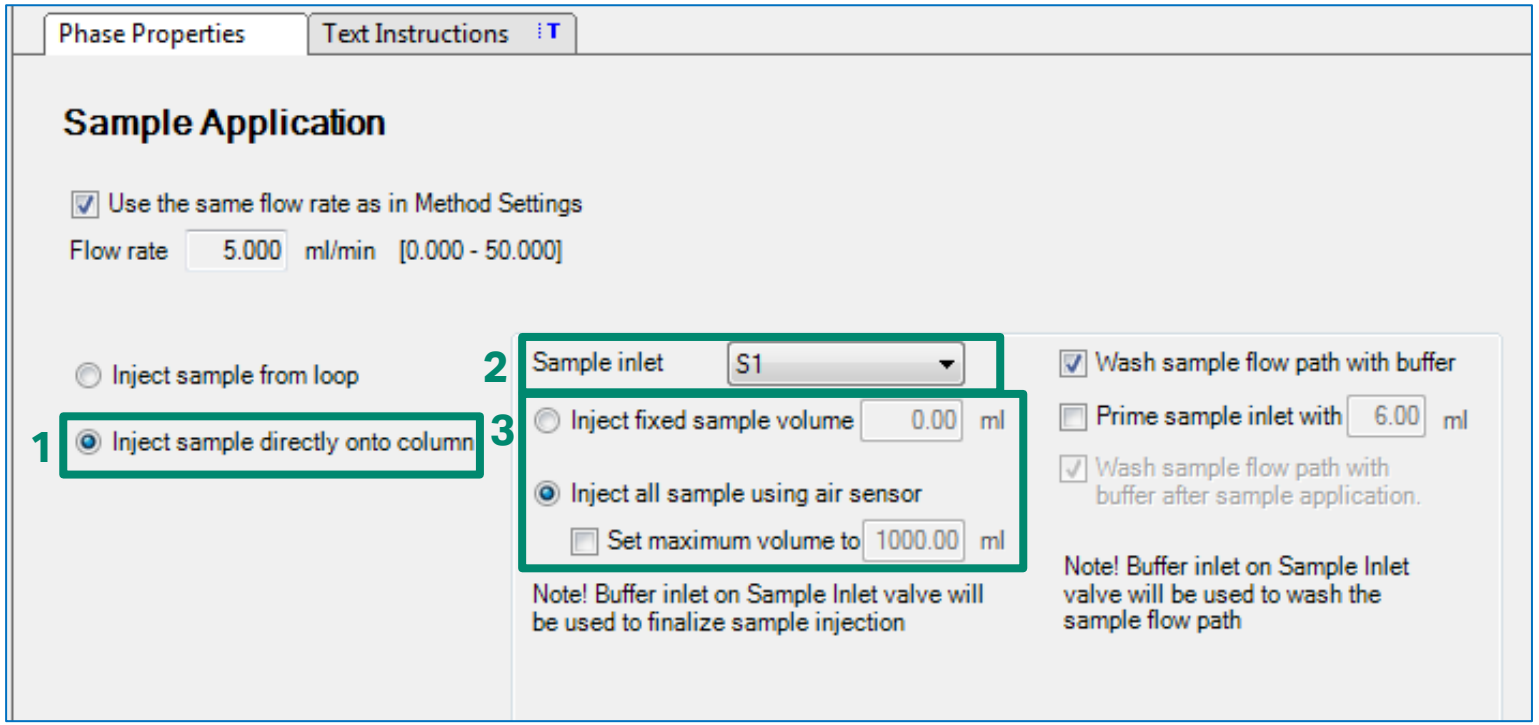
Edit the **Phase Properties** as appropriate.

Important: Make sure to select to inject sample directly onto the column in the **Sample Application** phase.

Important: Use the **peak to loop** option in **Elution** phase.

Note: Do not use Superloop™. The loop wash included in the method can cause overpressure and damage the Superloop.

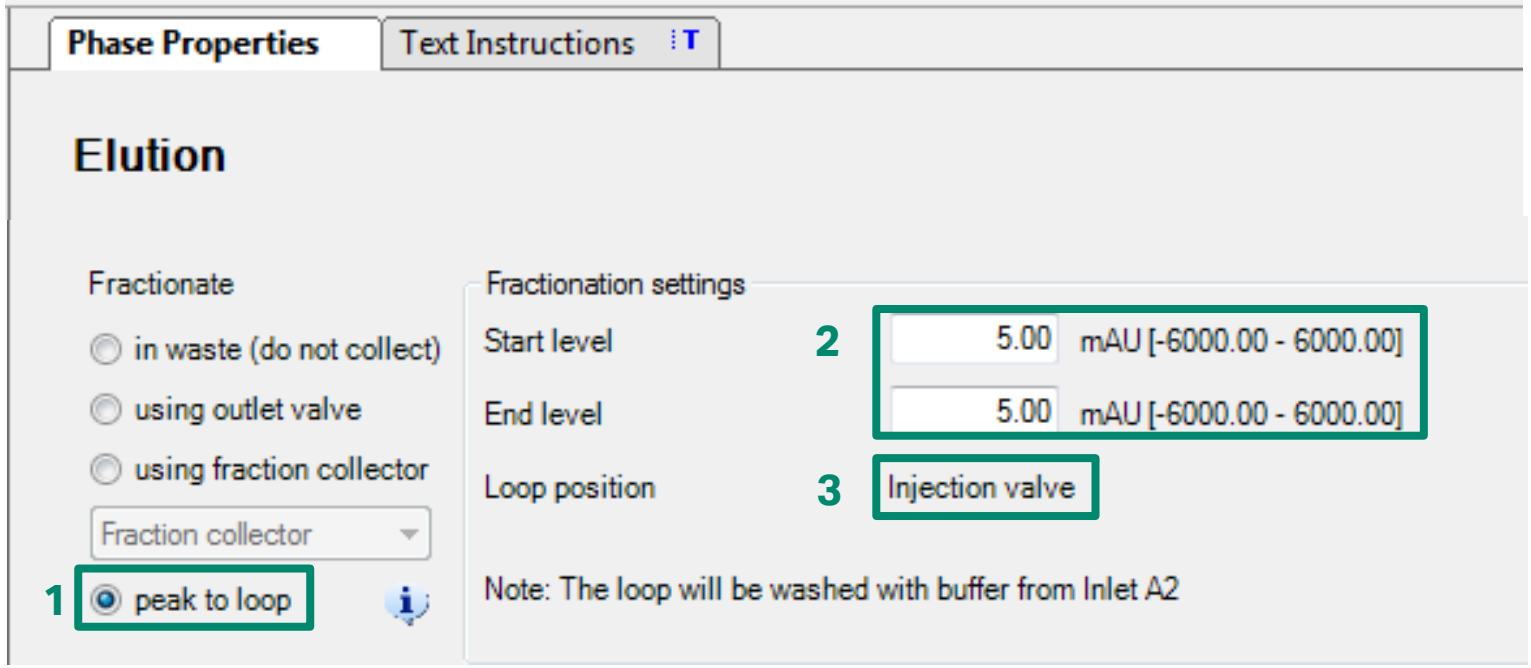
How to setup method for a capture step — sample application in the ÄKTA pure configuration including a sample pump



Adjust **Phase Properties** for **Sample Application**

1. Select to inject the sample directly onto column (the sample pump will be used).
2. Define which sample inlet to use.
3. Select whether to inject a fixed sample volume (define the volume) or use the air sensor and inject the entire sample.

How to setup the method for a capture step — elution in the ÄKTA pure configuration including a sample pump



Adjust **Phase Properties** for **Elution**

1. Select to collect the peak in a loop*.
2. Define UV levels for peak start and end identification.
3. The peak will be collected in the loop on the Injection valve**.

* Remember to place Inlet A2 in buffer container, will be used for loop wash.
**Remember to connect the Peak collect tubing from Out/Out 2 to SyR port on Injection valve.

Overview of the ÄKTA pure configuration with loop valve

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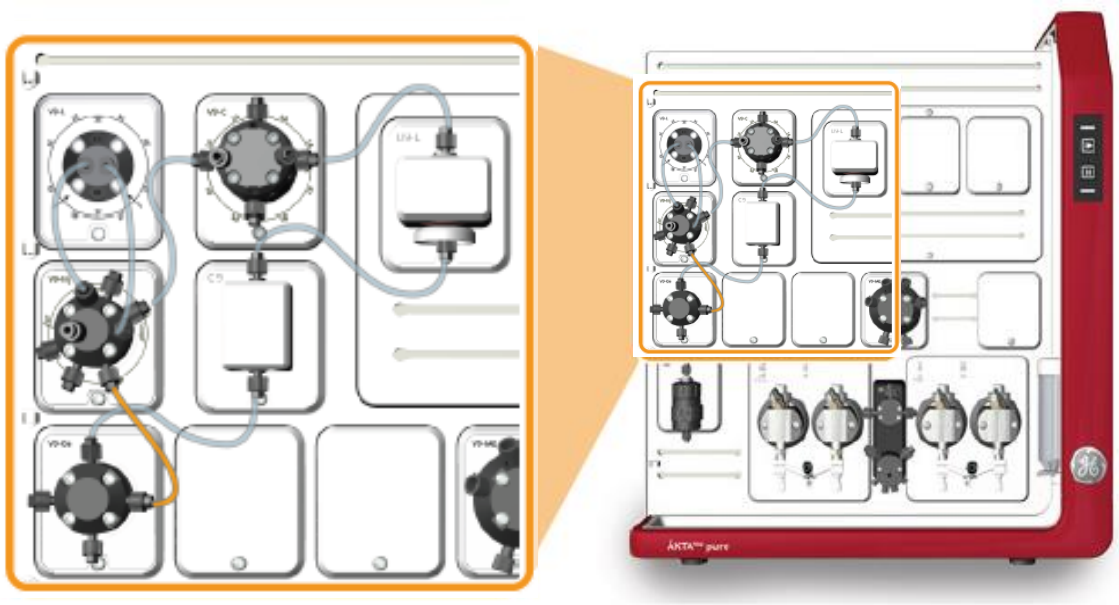
Two-step purification with intermediate peak collection in loop

ÄKTA™ pure configuration with loop valve



The intermediate peak is directed to the sample loop on the Loop valve before going to second column.

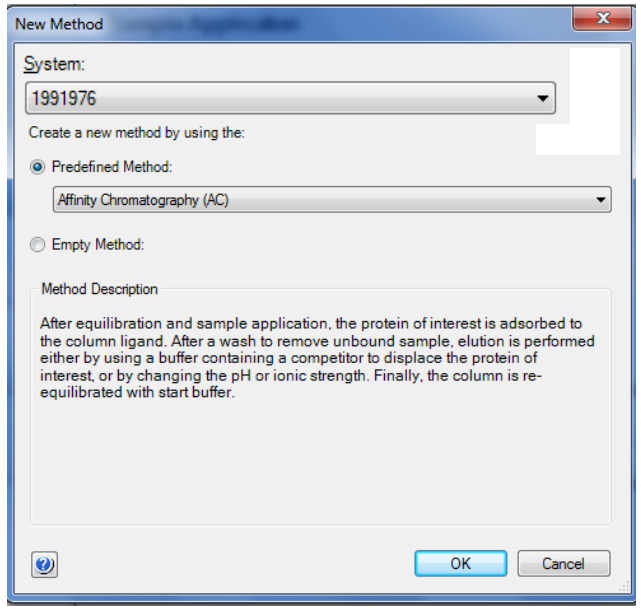
Required components

- Inlet valve V9-IA or V9-IAB
- Injection valve V9-Inj
- Loop valve V9-L
- Loop for sample loading in first step
- Tubing loop for collection of intermediate peak
- Column valve for 5 columns V9-C
- UV monitor U9-M or U9-L
- Outlet valve V9-O or V9-Os
- Peak collect tubing 25

Connect the Peak collect tubing from Out/Out 2 to SaP port on Injection valve.

How to setup method for a capture step in the ÄKTA pure configuration with loop valve

Select predefined method to be used



The Method Outline is displayed

Adjust Phase Properties

Edit the **Phase Properties** as appropriate.

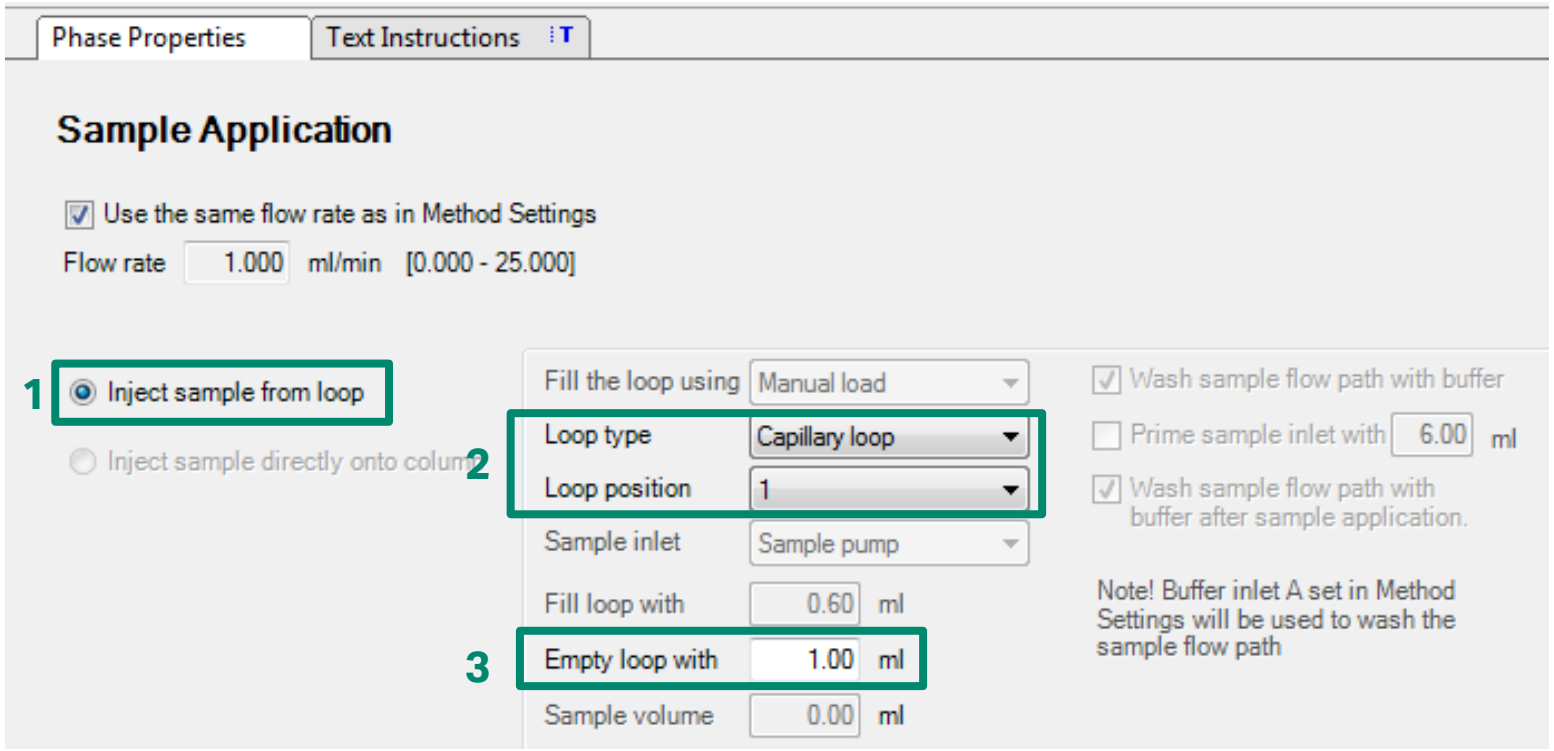
Important: Make sure to select to inject sample from loop and define the volume used to empty the loop with*.

Important: Use the **peak to loop** option in **Elution** phase.

Note: Do not use Superloop™. The loop wash included in the method can cause overpressure and damage the Superloop.

* To improve recovery, use excess volume of at least three times the loop volume to empty loop. If SEC is used, make sure that the allowed load volume is not exceeded.

How to setup method for a capture step — sample application in the ÄKTA pure configuration with loop valve

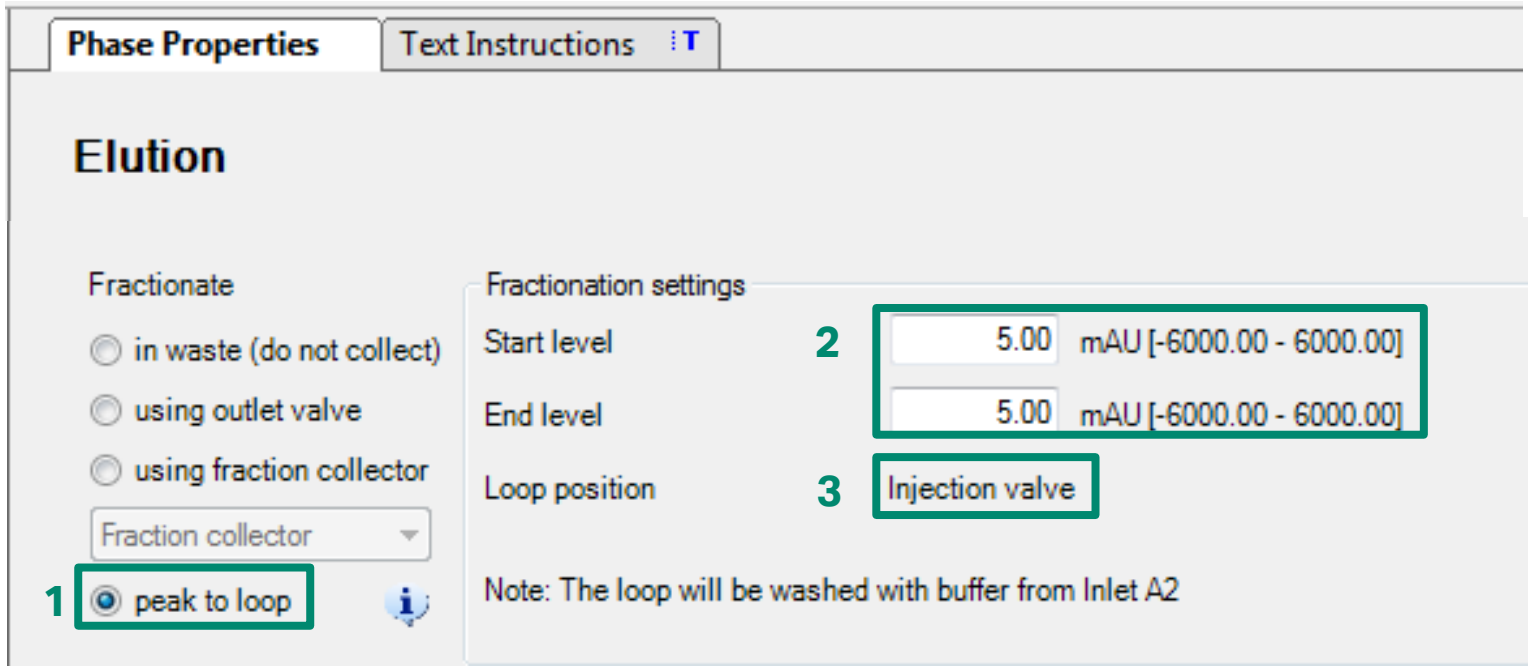


Adjust **Phase properties** for **Sample application**

1. Select to inject the sample from loop.
2. Select loop type: Capillary or Superloop™, and enter loop position to be used.
3. Define volume to empty loop with*.

* To improve recovery, use excess volume of at least three times the loop volume to empty the loop. If SEC is used, make sure that the allowed load volume is not exceeded.

How to set up the method for a capture step — elution in the ÄKTA pure configuration with loop valve



Adjust **Phase Properties** for **elution**

1. Select to collect the peak in a loop*.
2. Define UV levels for peak start and end identification.
3. The peak will be collected in the loop on the Loop valve**.

* Remember to place Inlet A2 in buffer container to be used for loop wash.
**Remember to connect the Peak collect tubing from Out/Out 2 to SaP port on Injection valve.

Advanced information

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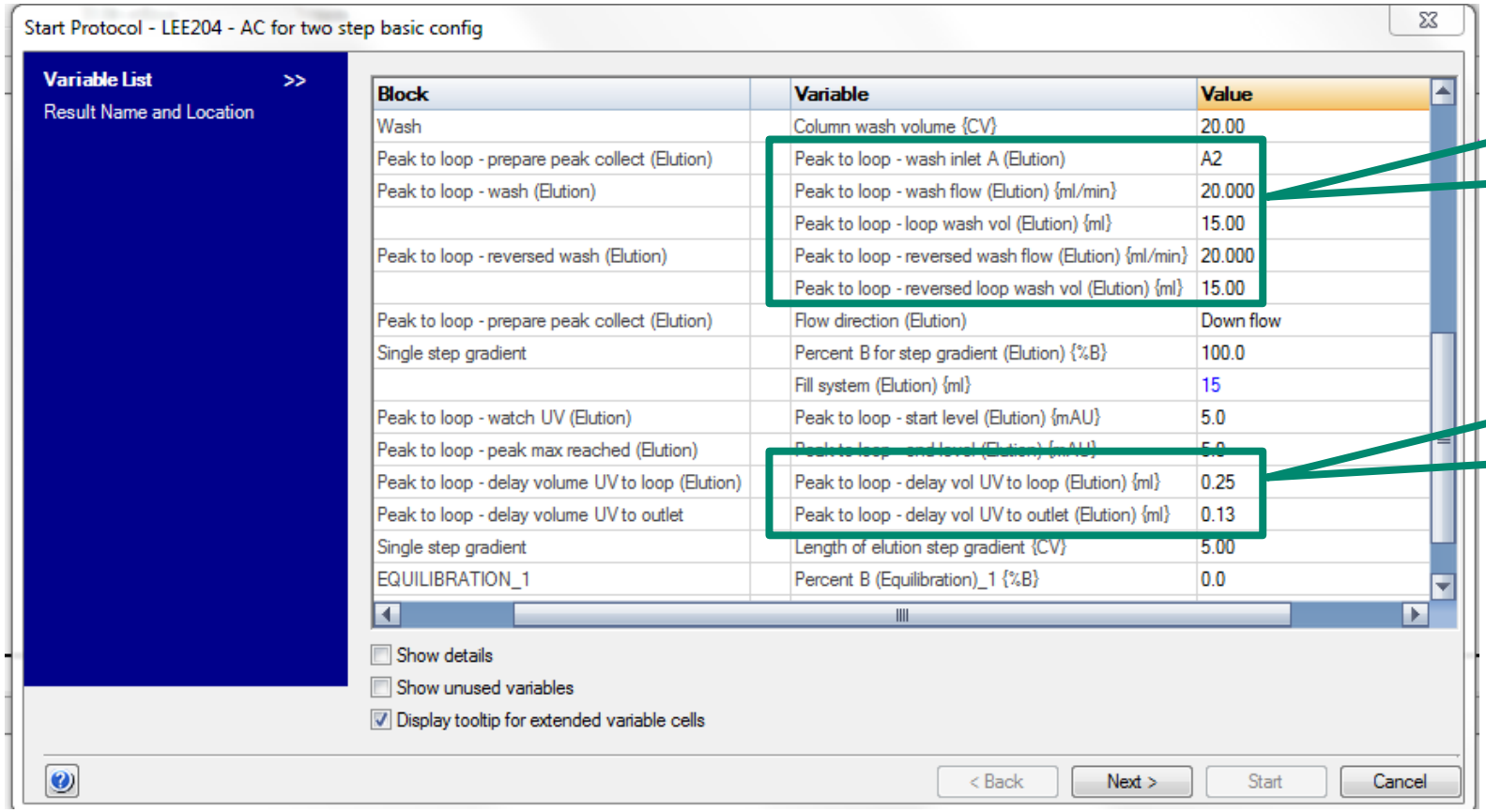
Questions and answers

What	Comment
Why is inlet A2 used to wash the loop during the capture step?	The method is assuming that inlet A2 will be used for the subsequent polishing step. Filling the loop with this buffer will ensure correct conditions for injection of the intermediate peak.
How many peaks can I collect during the elution step?	When the "Peak to loop" option is used, the first peak will be collected in the loop. Any subsequent peaks will be collected in outlet position "Waste" if V9-Os is used, or outlet position 10 if V9-O is used.
What columns sizes can I use?	The default flow path and methods are optimal for use of 1–5 mL columns in the capture step generating peak volumes of 1–5 mL. If the polishing step is SEC, HiLoad™ 16/60 and 26/60 columns can be used. **Note:** For improved recovery of intermediate peak, do not fill the loop with more than 70% of the loop volume. **Note:** Other column sizes can be used, however, depending on required recovery, the loop wash volumes and delay volumes might need adjustment (find description here).
Can I add other components to the set-ups described in this instruction?	Yes, additional components can be added. **Note:** Components added between UV and Outlet valve will change the delay volume affecting the recovery. See description on how to edit the delay volume used in the method.

Detailed information

What	Note
Loop wash in elution phase	This wash does NOT use the LoopWash instruction and does therefore not use the flow rate and volume defined by this instruction. The wash volume used by the method, is set to 30 mL (15 mL + 15 mL to wash in both directions) and buffer inlet A2 is used. The wash settings can be changed (find description here).
Delay volume for intermediate peak collection	Delay volume setting in System settings is NOT used. The volume is defined in the text method instead. The delay volume from UV to Outlet valve is assuming standard tubing and conductivity cell in line (0.13 mL). The delay volume from Outlet valve to Injection valve is based on the peak collect tubing kit (0.13 mL). The delay volume settings can be changed (find description here).
Sample pump set-up limitation	To be able to use the “Inject sample from loop” option, the sample pump needs to be removed from the components list and the peak collect tubing replumbed.
Outlet fractionation limitation	Set-up with V9-O (10 ports valve): position Out2 is occupied and cannot be used for outlet fractionation. Set-up with V9-Os: position Out is occupied and cannot be used for collection.

Display ***Start protocol*** at run start to change settings for loop wash and delay volume without using text editing



Loop wash volumes used in the elution phase can be changed here

Peak to loop delay volumes can be changed here

Thank you

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