Micro kit

Instructions for Use

Original instructions

1 Introduction

Overview

This document provides instructions on how to install the Micro kit on ÄKTA™ pure 25 M. This document also provides instructions on how to configure the UNICORN software and perform system performance test to ensure that the ÄKTA pure 25 M with the installed Micro kit functions properly.

The following components are required to setup the ÄKTA pure 25 M for microscale purification using the Micro kit:

- ÄKTA pure 25 M
- Micro kit
- UNICORN™ software
- Fraction collector F9-R (if fraction collection is to be performed)

Read this before operating the product

All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the ÄKTA pure *Operating Instructions 29022997* at hand when operating the product.

Do not install, operate, or perform maintenance on the product in any other way than described in the user documentation. If you do, you may be exposed or expose others to hazards that can lead to personal injury and you may cause damage to the equipment.

2 Description

Intended use

The Micro kit is intended for use on ÄKTA pure 25 M with multiple wavelenght monitor U9-M. It will improve the performance of the instrument and allow microscale purification. To enable collection of fractions in microliter scale it is recommended to use the fraction collector F9-R in combination with the Micro kit.

Configuring the ÄKTA pure 25 M for microscale purification with the Micro kit involves moving and/or replacing components as described in this document. All the components can be installed by the end users and if required the instrument can at a later point be restored to its original configuration.

Components included in the Micro kit

- Injection valve **V9M-J**
- UV flow cell **U9-2M**
- Conductivity monitor **C9M**
- Outlet valve **V9M-Os**
- Online filter
- Mixer chamber 0.6 mL
- Tubing kit
- 1/16 in male-male union and i.d. 0.25 mm fingertight union
- 1/16 in male-male union and i.d. 0.13 mm fingertight union
- Micro nozzle for fraction collector **F9-R**
- Multidirectional column clamp
- Sample loops (10µl and 50µl)
- Injection kit
- Tube holders för Eppendorf tubes

ÄKTA pure 25 M components used together with the Micro kit

- Pumps **P9** (2x)
- Pressure monitor **R9**
- Mixer **M9**
- UV monitor **U9-M** with detector **U9-D**
- Flow restrictor FR-902

Optional modules

The following optional modules supported by ÄKTA pure 25 M can be used for micro-scale purification:

- Fraction collector **F9-R**
- Air sensor **L9-1.5** placed before pumps
- Column valve **V9-Cs**
- Alias™ Bio autosampler connected according to *Instruction 29040427*

Note: *Column valve or autosampler adds extra volume that could decrease resolution for some columns.*

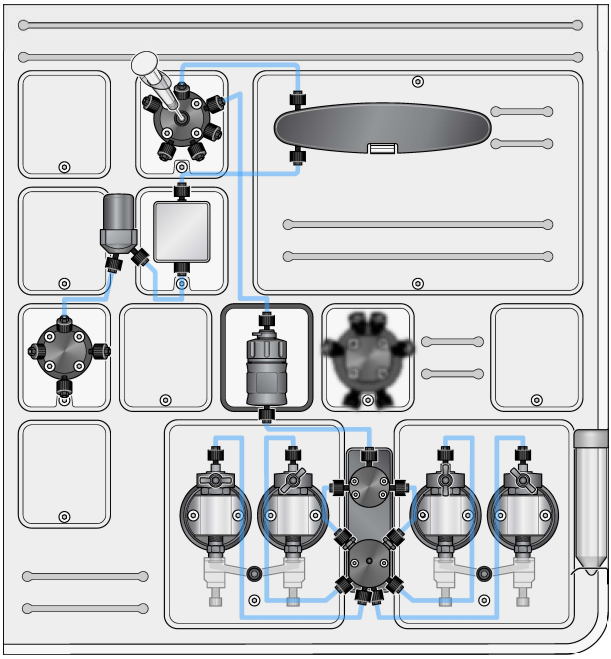
Note: *Other modules not listed here but supported by ÄKTA pure 25 M are not recommended for micro-scale purification due to large internal volumes that will decrease system performance, e.g. reduced resolution and increased peak broadening.*

Tubing kits

- Pre-injection tubing, from pumps to injection valve.
- Blue kit. Post-injection tubing, for flow rates 0.25- 2 mL/min.
- Red kit. Post-injection tubing, for flow rates ≤ 0.25 mL/min.

Setup on ÄKTA pure 25M

The illustration below shows ÄKTA pure 25 M when the Micro kit is installed (without optional modules). This configuration for micro-purification optimizes the flow path and keeps dead volume to a minimum.



3 Installation

3.1 Included steps

Step	Action
1	**Performance test 1**: To make sure that the system is running properly before changing configuration.
2	**Install Micro kit components**: Includes removal and replacement of hardware modules.
3	**Install tubing kit** Connect the tubing in appropriate positions.
4	**Configure software** Adjust system properties and settings in UNICORN.
5	**Test the installation** To make sure that all tubing and connectors are installed properly.
6	**Performance test 2** To make sure that the system is running properly after installation of the Micro kit.

3.2 Performance test 1

System performance test are built-in methods to check the functionality and performance of the ÄKTA system. Make sure the system is fully functional by running a test before installing the Micro kit and one after the kit installation is completed.

For information how to run a system performance test, see the User Manual 29119969.

Before installing the Micro kit run the following tests:

- System test with UV U9-M (variable)
- Optional: Fraction collector F9-R test

For test after the installation, see *Section 3.7 Performance test 2, on page 18*.

3.3 Install the Micro kit components

Introduction

For information on how to install a module see *ÄKTA pure User Manual 29119969*

Make sure to run a system performance test as described in *Installation, on page 6*.

WARNING

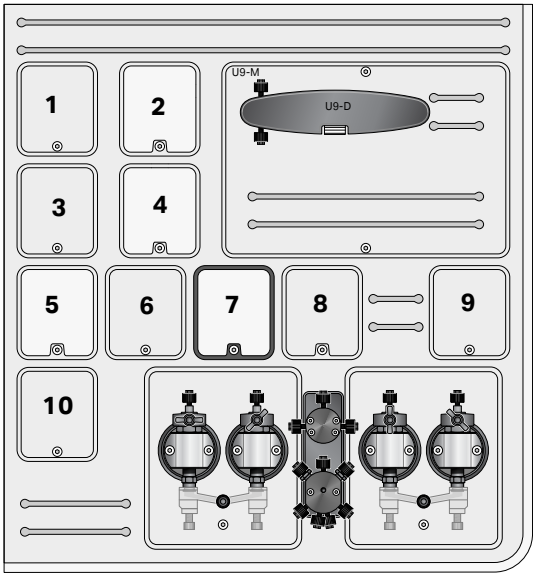
Disconnect power Always switch off power to the ÄKTA system before replacing any of its components, unless stated otherwise in the user documentation.

WARNING

Hazardous chemicals or biological agents.Make sure that the entire flow path has been flushed thoroughly with bacteriostatic solution (e.g., NaOH) and distilled water, before service and maintenance.

Module positions

To be able to use the pre-cut tubing and achieve the lowest internal volumes the Micro kit modules must be placed in position 2, 4, 5 and 7 according to the illustration below. All unused positions require a Module panel (dummy module) or an unused module with the cable connected.



Module node ID

The Micro kit modules are delivered pre-configured with the correct node ID. If there are connection issues check the node-ID of the modules, see *Section 5.6 Node IDs, on page 32*.

Remove tubing

Remove all PEEK tubing and outlet tubing. Leave the waste tubing and inlet tubing (before pumps).

Move the mixer

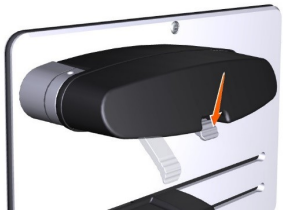
Step	Action
1	Remove the Module panel in position 7.
2	Move the Mixer **M9** to position 7 and install a Module panel in position 10.
3	Attach the 0.6 mL mixer chamber.

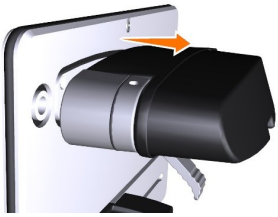
Replace the injection valve

Step	Action
1	Remove the Module panel in position 2.
2	Disconnect the waste tubing **W1** and **W2** from the injection valve **V9-Inj**.
3	Remove the injection valve **V9-Inj** and install a Module panel in its position.
4	Install the injection valve **V9M-J** in the position 2.
5	Connect the waste tubing **W1** and **W2** on the new valve to ports **W1** and **W2**.

Replace the UV flow cell

Step	Action
1	Push the latch on the UV detector to disconnect the detector.



Step	Action
2	Remove the detector and the flow cell from the monitor. Be careful not to damage the UV flow cell by pulling too hard.  ***Note:*** *Make sure that the flow cell does not come into contact with any liquid, and that no liquid enters the UV detector or monochromator.* ***Note:*** *Do not touch the optical fiber connectors as this will result in poor monitor performance. If you accidentally touch the optical fiber connectors, clean them according to the User Manual 29119969.*
3	Disconnect the UV flow cell from the UV detector. ***Note:*** *While the UV detector is disconnected, protect the fiber connectors from dust or other impurities with the protective caps.*
4	Remove one of the black protective caps from a new UV flow cell and connect the cell to the detector.
5	Remove the remaining black cap and connect the detector with the new flow cell to the monitor.
6	Pull the latch upwards to fasten the detector.

Replace the conductivity monitor

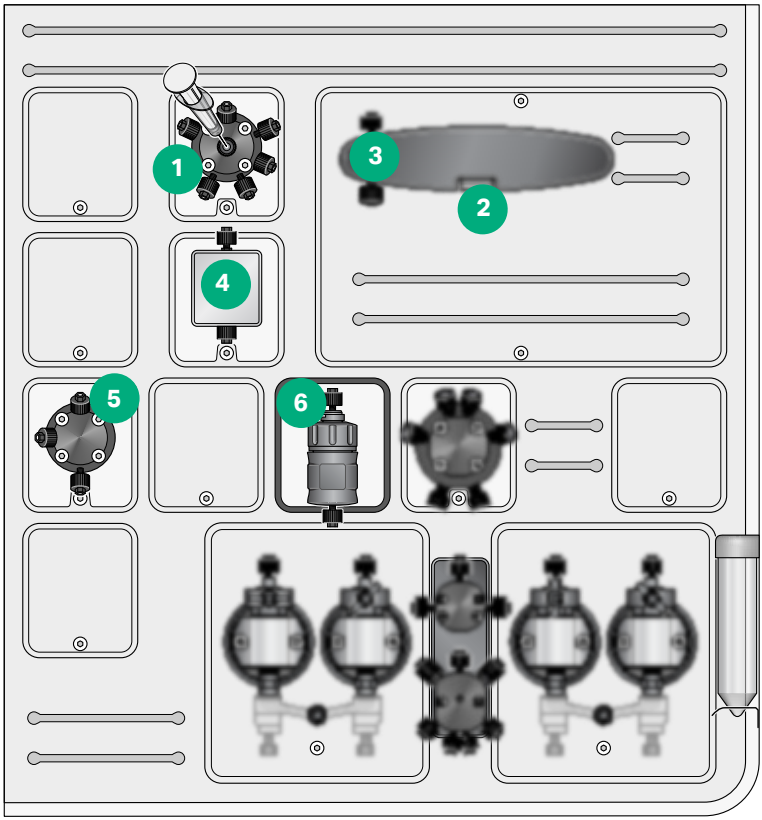
Step	Action
1	Remove the Conductivity monitor **C9n**.
2	Move the flow restrictor **FR-902** and the holder (including the screw) to the new conductivity monitor **C9M**.
3	Install the new Conductivity monitor **C9M** in position 4.

Replace the outlet valve

Step	Action
1	Disconnect the waste tubing W from the Outlet valve.
2	Remove the Outlet valve.
3	Install the new Outlet valve **V9M-Os** in position 5.
4	Connect the waste tubing W on the new valve port **W**.

Final check

Check that the modules are installed as in the illustration below. All positions must have a module or a Module panel with the cable connected.



Positions of the module panel

The table below shows the positions of the module panel.

Label	Component	Description
1	V9M-J	Injection valve
2	U9-M	UV monitor
3	U9-2M	UV flow cell
4	C9M	Conductivity monitor
5	V9M-Os	Outlet valve
6	M9	Mixer 0.6 mL

3.4 Install the tubing kit

Description of the tubing kit

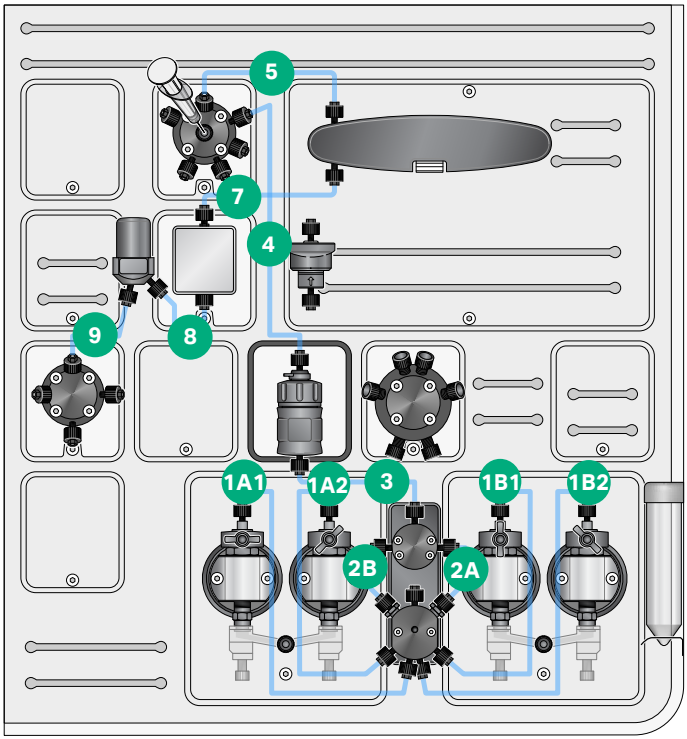
- Pre-injection tubing, from pumps to injection valve. PEEK “blue”, i.d. 0.25 mm.
  - “blue" kit. PEEK blue, i.d. 0.25 mm, post-injection tubing. For flow rates 0.25 - 2 mL/min. E.g. for columns Capto HiRes or 10/300 SEC.
  - “red” kit. PEEK red, i.d. 0.13 mm, post-injection tubing. For flow rates ≤ 0.25 mL/min. E.g. for SEC columns 3.2/300 and 5/150.

The "red" tubing with i.d. 0.13 mm will give the best performance in terms of resolution. It will however generate more back pressure in the system which is why the blue tubing with i.d. 0.25 mm is recommended for higher flow rates.

Note: *Do not install a column yet. No column must be included when running the system performance test.*

Tubing installation

Use the blue tubing kit, this is required for the system performance test to be run after the installation. Start the installation of tubing from the pump and continue with the tubing in numerical order. Make sure the tubing are installed as in the illustration below.



"Blue" tubing kit

The table below shows the components of the "Blue" tubing kit.

Label	Description
1A1	Pump head to pump restrictor (left side)
1A2	Pump head to pump restrictor (left side)
1B1	Pump head to pump restrictor (right side)
1B2	Pump head to pump restrictor (right side)
2A	Pump restrictor (right side) to Pressure monitor (right side)
2B	Pump restrictor (left side) to Pressure monitor (left side)

Label	Description
3	Pressure monitor to Mixer 0.6ml
4	Mixer 0.6 mL to Injection valve **V9M-J** (port Pump)
5	Injection valve **V9M-J** (port Col) to Column or directly to UV monitor for running Performance test
7	UV monitor **U9-M** to Conductivity monitor **C9M**
8	Conductivity monitor **C9M** to Flow restrictor (port IN)
9	Flow restrictor to Outlet valve **V9M-Os** (port In)
Frac	Outlet valve **V9M-Os** (port Frac) to Fraction collector nozzle

For more details on tubing ses *Section 5.3 Flow path and tubing, on page 28*.

Note: *Connect tubing "5" directly to the UV flow cell when running the system performance test.*

Note: *Use fresh fingertight connectors. Tighten by hand and then use the wrench to further tighten ca 1/8 of a turn.*

Note: *Check that all waste tubing (W, W1, W2) are connected as described in* *Section 3.3 Install the Micro kit components, on page 7*

Note: *Connect one of the supplied sample loops to the injection valve **V9M-J** (ports LoopE and LoopF).*

3.5 Install and configure the software

Download the instrument configuration

The ***Instrument Configurations*** is available for free download from the ÄKTA pure 25 product page on the website www.cytivalifesciences.com. Go to Related documents - Software - ÄKTA pure 25 Instrument Configuration software. Download the zip file.

Note: *The version must be 1.13.0.61 or later.*

Import the instrument configuration

The ***Instrument Configurations*** must be imported into the database for the system.

Step	Action
1	Select the ***Tools*** menu in the ***Administration*** module in UNICORN.
2	Click ***SystemProperties*** or click the ***SystemProperties*** icon to open the dialog.
3	Click ***Instrument Configurations***.
4	Click ***Import*** on the ***InstrumentConfigurations*** dialog and select the downloaded zip file.
5	Click ***Close***.

Select and configure the instrument configuration

The ***System Properties*** must be updated for the Micro kit.

Note: *Instrument modules are referred to as* ***Components*** *in UNICORN*

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Step	Action
1	Go to ***System Properties***.
2	Select the system in the ***System Properties*** dialog.
3	Click ***Edit***.
4	Select the relevant instrument configuration in the ***Instrument configuration*** dropdown menu.
5	Go through all ***Component types*** and select the installed modules.

Step	Action
6	Select Injection valve, with property **V9M-J**.
7	Select Outlet valve, with property **V9-Os** (1-outlet). ***Note:*** ***V9M-Os*** *is not listed but selected as V9-Os.* ***Note:*** *Do not forget to select Conductivity monitor.* ***C9M*** *is not listed but selected as* ***C9****.* ***Note:*** *All installed modules, not only new modules, must be selected or unselected for not installed modules..*
8	Click ***OK*** to apply the changes

Connect to the system

To ensure the connection between the ÄKTA pure instrument and UNICORN perform the following:

- Turn on the power of the ÄKTA pure instrument.
- In System control connect to the system.

Note: *If system connect fails check the following:*

- *All installed modules are selected in UNICORN as described in Select and configure the instrument configuration, on page 16 (including non-used modules).*
- *Node ID of all installed modules is correct as described in Section 5.6 Node IDs, on page 32.*
- *Restart the computer and log on to UNICORN.*

Adjust System Settings

Some changes of ***System Settings*** are needed after installation of the Micro kit.

Tubing and delay volumes settings

Select in ***UNICORN*** →***System Control*** →***System*** →***Settings*** →***Tubing and Delay volumes***.

- Set ***Tubing: Injection valve to Column*** to i.d. 0.25 mm and length 520 mm
- Set ***Delay volume: Monitor to outlet valve*** to 38 µL

- Set ***Delay volume: Monitor to frac*** to 60 µL

Note: *Change these values according to table in Section 5.5 Delay volumes, on page 30 if the kit with red tubing i.d. 0.13 mm is used.*

Wash settings

Select in ***UNICORN*** →***System Control*** →***System*** →***Settings*** →***Wash settings***.

- Set ***System wash flow rate*** to 10 mL/min
- Set ***System pump wash flow rate*** to 10 mL/min
- Set ***System pump wash volume*** to 10 mL

Note: *Adjust the flow rate during the system wash to avoid high system pressure if a column valve is used.*

Fractionation setting

When using the fraction collector **F9-R** change the following.

Select in ***UNICORN*** →***System Control*** →***System*** →***Settings*** →***Fraction collection*** →***Fractionation setting*** and set **Drop Sync** to **ON**.

3.6 Test the installation

Follow the steps below to make sure that all tubing and connectors are installed properly, before proceeding to performance test 2.

Step	Action
1	Prime inlets A1 and B1 with water.
2	Purge the pumps.
3	Start a flow of 1 mL/min.
4	Check for leakage. The pressure shall be stable and above 0.2 MPa.
5	Change to 100 % B and repeat step 4.

3.7 Performance test 2

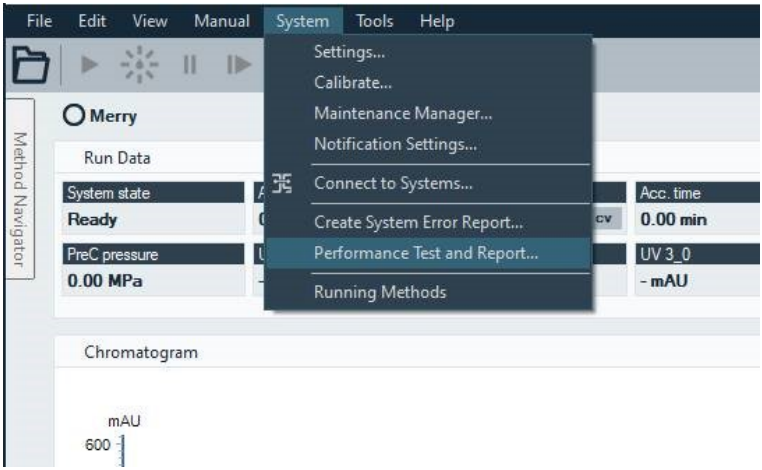
After installing the Micro kit and adjusting the software settings run the system performance test Micro kit.

The system test assumes that the system is configured with:

- 0.6 mL mixer
- Flow restrictor FR-902
- “Blue” tubing kit i.d. 0.25 mm

- Tubing “5” connected directly to the UV flow cell, i.e. no column, see *Tubing installation, on page 14*.

The screenshot below shows the location of the System Performance Test in UNICORN.



4 Considerations for running micro- scale purification

4.1 Columns

Connect a column

To increase the resolution, it is recommended to connect the column directly to the UV monitor using a male-male connector. Both i.d. 0.25 mm and i.d. 0.13 mm are supplied. To secure the column, use the supplied multidirectional column clamp.

Note: *With the Micro kit configuration, the gain in volume by using horizontal orientation of the column is insignificant (~1 µL).*

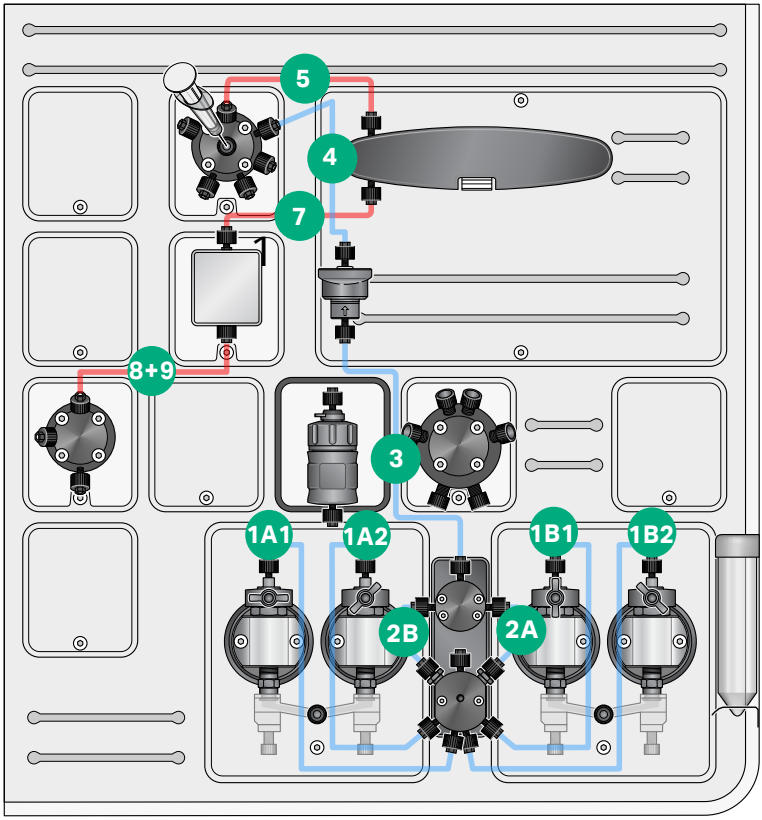
Select tubing kit

Use the "red" i.d. 0.13 mm tubing kit for columns of type 3.2/300 and 5/150. For all other Cytiva pre-packed columns the "blue" i.d. 0.25 mm tubing is recommended. In general, the "red" i.d. 0.13mm tubing kit is recommended for low flow rates up to 0.25 mL/min. With higher flow rates, the generated back pressure will be too high.

Note: *Remember that the pressure generated by tubing increases by 16x if the i.d. is halved. On the other hand, the volume is increased by 4x if the i.d. is doubled.*

Note: *The flow restrictor FR-902 is not needed with the "red" tubing kit. See* *Section 4.2 Pressure and pressure limits, on page 23* *for more information.*

The illustration below shows the “red” tubing kit. See *Tubing installation, on page 14* for an illustration of the “blue” tubing kit.



"Red" tubing kit

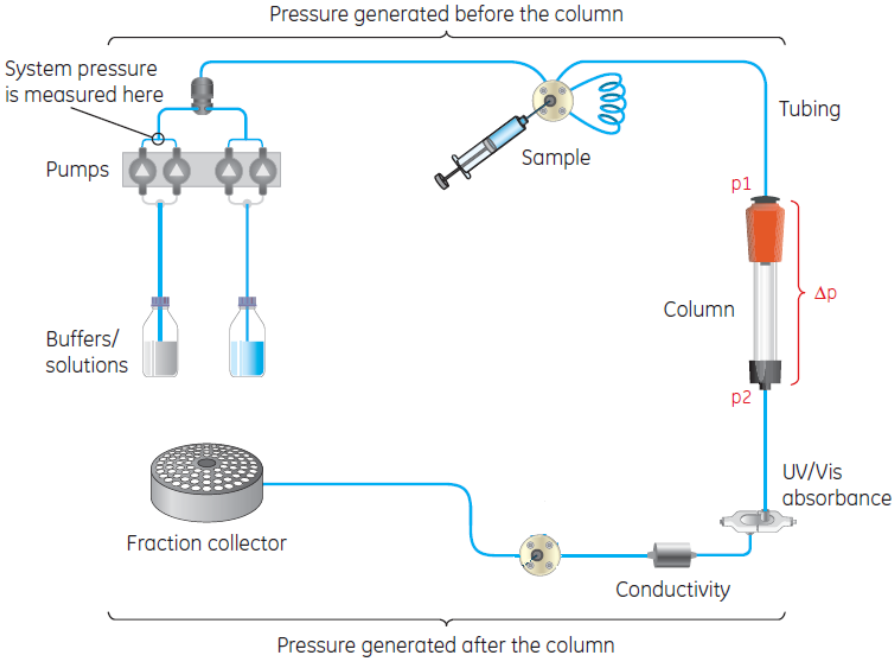
The table below shows the components of the "Red" tubing kit.

Label	Tubing	Length	Description
1A1	PEEK I.D. 0.25 mm	340 mm	Pump head to pump restrictor (left side)
1A2	PEEK I.D. 0.25 mm	340 mm	Pump head to pump restrictor (left side)
1B1	PEEK I.D. 0.25 mm	340 mm	Pump head to pump restrictor (right side)

Label	Tubing	Length	Description
1B2	PEEK I.D. 0.25 mm	340 mm	Pump head to pump restrictor (right side)
2A	PEEK I.D. 0.25 mm	115 mm	Pump restrictor (right side) to Pressure monitor (right side)
2B	PEEK I.D. 0.25 mm	115 mm	Pump restrictor (left side) to Pressure monitor (left side)
3	PEEK I.D. 0.25 mm	160 mm	Pressure monitor to Online filter
4	PEEK I.D. 0.25 mm	250 mm	Online filter to Injection valve V9M-J (port Pump)
5	PEEK I.D. 0.13 mm	520 mm	Injection valve V9M-J (port Col) to Column
(6)	M-M connector	N/A	Column to UV monitor U9-M
7	PEEK I.D. 0.13 mm	170 mm	UV monitor U9-M to Conductivity monitor C9M
8+9	PEEK I.D. 0.13 mm	175 mm	Conductivity monitor C9M to Outlet valve V9M-Os (port In)
Frac	PEEK I.D. 0.13 mm	400 mm	Outlet valve V9M-Os (Frac) to Fraction collector nozzle

4.2 Pressure and pressure limits

Description of pressure measurement



System pressure, generated by the complete system flow path, is measured in ÄKTA chromatography systems at the system pump. Δp = p1 (pressure generated after and by the column itself) – p2 (pressure generated after the column).

The pressure at the pump, System pressure, is always the highest pressure in the system. It can be divided into different part as:

$P_{system} = P_{before} + P_{delta\text{-}column} + P_{after}$

The pressure values related to columns are defined as:

$P_{pre\text{-}column} = P_{delta\text{-}column} + P_{after}$

$P_{post\text{-}column} = P_{after}$

$P_{delta\text{-}column} = P_{pre\text{-}column} - P_{post\text{-}column}$

The $P_{pre\text{-}column}$ value needs to be monitored to protect the column hardware. The $P_{delta\text{-}column}$ value needs to be monitored to protect the packed resin.

Setting pressure alarms

Only the $P_{pre\text{-}column}$ pressure alarm can be set in UNICORN when the column valve V9-C with pressure sensors is not installed. To protect the packed bed, the $P_{delta\text{-}column}$ pressure limit from the column list needs be used and set as $P_{pre\text{-}column}$ pressure alarm value. This is done automatically in UNICORN for predefined methods or phases for most glass columns.

Pressure measurement with the Micro kit

Without the column valve **V9-C** with built-in pressure sensors only the system pressure is measured.

The $P_{pre\text{-}column}$ pressure is based on:

- Psystem
- Flow rate
- Tubing length
- Temperature

The $P_{pre\text{-}column}$ pressure value is calculated based on the system pressure and the pressure drop between the system pressure sensor and the column. Make sure to enter the value of the tubing dimensions from the injection valve to the column in the System settings, see *Adjust System Settings, on page 17*.

Note: *For the "red" i.d. 0.13 mm tubing kit since the correct i.d. cannot be set. use 0.25 mm instead. To get a more accurate calculation, increase the value for length. Use 8000 mm as a starting point. Test the pressure calculation by running at the maximum recommended flow rate 0.25 mL/min with the end of tubing position 5 open (not connected anywhere). The displayed pre-column pressure should be near 0. Decrease the value for length if the pressure is negative.*

Flow restrictor

A flow restrictor creates a steady back pressure that keeps the air dissolved in the solution. Air bubbles formed after the column, due to pressure dropping, might disturb the detector signal.

The flow restrictor back pressure (0.2 MPa) only affects the column hardware pressure whereas the pressure on the packed bed is unaffected.

It is recommended to always keep the flow restrictor inline. However, when using the "red" i.d. 0.13 mm tubing kit there is enough pressure without the flow restrictor. To avoid unnecessary peak broadening it is recommended to exclude the flow restrictor when using the red tubing kit.

4.3 Injection techniques

Manual sample injection

For good chromatographic resolution on SEC columns in analytical or small-scale preparative work, the sample volume should not exceed 2% of the column volume. When connecting a small loop and working with complete loop filling techniques, the total sample volume is the sum of the loop volume and the loop connecting channels in the valve. The micro injection valve V9M-J has low internal volume to minimize extra sample load. Remember not to remove the syringe before the valve has turned.

When loading small sample volumes into injection valve, use the Fill port in the Injection kit INV-907 which is made for syringes with needle o.d. 0.7 mm. Make sure that the needle is blunt ended.

Note: *Do not use Luer connection since that is designed for larger volumes.*

Manual complete filling

The micro injection valve **V9M-J** has minimal channel contribution to the loop volume.

Due to laminar flow, it is recommended to fill with an excess volume of sample that corresponds to 3 to 5 times the loop- and valve-volume. This recommendation is a general recommendation for all small SEC columns irrespective of the loop size chosen. Correspondingly, when injecting the sample into the column, empty the loop and the injection valve channels with an excess of buffer.

Sample injection using Alias autosampler

Manual partial filling requires experience with liquid chromatography systems and a more reliable method is to use an Alias autosampler.

The recommended volumes for manual loop filling also applies when using the Alias autosampler, both for complete and partial filling. Partial filling of a small loop, for example 1 µL in a 10 µL loop, is a way to further increase the resolution.

For more information about installing and operating the autosampler see, Connect Alias autosampler to ÄKTA pure, article number 29040427.

4.4 Gradients

The size of the mixer compared to the length of the gradient is very important since a too large mixer will distort short gradients. The following table shows general recommendation for the Micro kit. A larger mixer may be needed when creating gradients with some solvents.

Flow rate	Mixer size
0.25 – 2 mL/min	0.6 mL

Flow rate	Mixer size
0.05 – 0.25 mL/min	No mixer

Note: *If running without a mixer, it is recommended to use the online filter in its place between the pressure monitor and the injection valve.*

4.5 Fraction collection

The fraction collector **F9-R** is recommended for use with the Micro kit.

Note: *For collecting small fraction use the supplied tube holders with Eppendorf™ tubes.*

The micro outlet valve V9M-Os has minimal volume and do not need to by-passed to achieve low band-broadening in the fraction collector. Use short tubing length between the outlet valve and the fraction collector. Pre-cut tubing of 400 mm is supplied which is appropriate for placing the fraction collector next to the ÄKTA pure system. Remember to set the appropriate delay volume, see *Section 5.5 Delay volumes, on page 30*.

Creating small drops

With water-based liquids at room temperature, the drop size with the micro nozzle is ~8 µL. The micro nozzle, including the use of Drop Sync, is recommended when using the "red" tubing kit with flow rates up to 0.25 mL/min.

5 Reference information

5.1 System specifications

Most specifications of ÄKTA pure 25 are still valid after installation of the Micro kit. The following values differ.

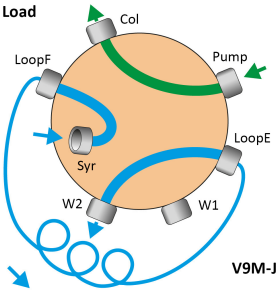
Note: *Flow rate up to 25 mL/min is still available but not recommended due to excessive pressure.*

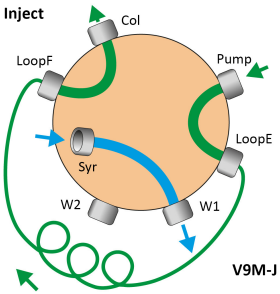
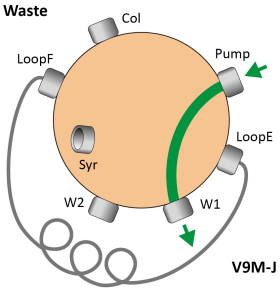
The table below shows the specifications of the ÄKTA pure 25 with the Micro kit.

Recommended flow rate range	0.01 – 2 mL/min
Recommended gradient flow rate range	0.25 – 2 mL/min with mixer 0.6 mL 0.05 – 0.25 mL/min without mixer
Gradient linearity range	5 - 90 %B at flow rates 0.25 - 2 mL/min with mixer 0.6 mL 10 - 90 %B at flow rates 0.05 - 0.25 mL/min without mixer
UV flow cell U9-2M	Optical path length 2 mm Illuminated volume 0.8 µL Total volume: 2.6 µL

5.2 Injection valve

The table below show the function of the micro injection valve V9M-J.

Position	Flow path illustration	Description
Load		The flow is directed onto the column of column valve. Sample can be loaded manually into loop through the syringe port **Syr**. Excess sample leaves through the waste port **W2**.

Position	Flow path illustration	Description
Inject		The flow is directed through the loop and onto the column or column valve. The syringe port can be washed manually in this position.
Waste		The flow is directed to waste through the **W1** port. This flow path is used for performing a pump wash.

5.3 Flow path and tubing

Tubing kit 1

The table below shows the 1, pre-injection of the tubing kit.

Label	Tubing	Length	Description
1A1	PEEK "blue" I.D. 0.25	340 mm	Pump head to pump restrictor (left side)
1A2	PEEK "blue" I.D. 0.25	340 mm	Pump head to pump restrictor (left side)
1B1	PEEK "blue" I.D. 0.25	340 mm	Pump head to pump restrictor (right side)
1B2	PEEK "blue" I.D. 0.25	340 mm	Pump head to pump restrictor (right side)
2A	PEEK "blue" I.D. 0.25	115 mm	Pump restrictor ((left side) to Pressure monitor
2B	PEEK "blue" I.D. 0.25	115 mm	Pump restrictor (right side) to Pressure monitor
3	PEEK "blue" I.D. 0.25	160 mm	Pressure monitor to Mixer
4	PEEK "blue" I.D. 0.25	250 mm	Mixer to Injection valve

Tubing kit 2a "blue"

The table below shows the post-injection, for flow rates 0.25 - 2 mL/min of the "blue" tubing kit 2a.

Label	Tubing	Length	Volume	Description
5	PEEK I.D. 0.25 mm	520 mm	26.3 µL	Injection valve V9M-J (port Col) to Column
(6)	M/M union i.d. 0.25 mm		2,0 µL	Column to UV monitor U9-M
7	PEEK I.D. 0.25 mm	170 mm	8.6 µL	UV monitor U9-M to Conductivity monitor C9M
8	PEEK I.D. 0.25 mm	95 mm	4.8 µL	Conductivity monitor to Flow restrictor (port IN)
9	PEEK I.D. 0.25 mm	135 mm	6.8 µL	Flow restrictor to Outlet valve V9M-Os (port In)
Frac	PEEK I.D. 0.25 mm	400 mm	20.3 µL	Outlet valve V9M-Os (port Frac) to Fraction collector nozzle

Tubing kit 2b "red"

The table below shows the post-injection, for flow rates ≤ 0.25 mL/min of the "red" tubing kit 2b.

Label	Tubing	Length	Volume	Description
5	PEEK "red" I.D. 0.13 mm	520 mm	6.6 µL	Injection valve V9M-J (port Col) to Column
(6)	M/M union i.d. 0.13 mm		0.5 µL	Column to UV monitor U9-M
7	PEEK I.D. 0.13 mm	170 mm	2.2 µL	UV monitor U9-M to Conductivity monitor C9M
8+9	PEEK I.D. 0.13 mm	175 mm	2.2 µL	Conductivity monitor C9M to Outlet valve V9M-Os (port In)
Frac	PEEK I.D. 0.13 mm	400 mm	5.1 µL	Outlet valve V9M-Os (port Frac) to Fraction collector nozzle

5.4 Component volumes

The table below shows the flow path volumes of the modules and components in the Micro kit.

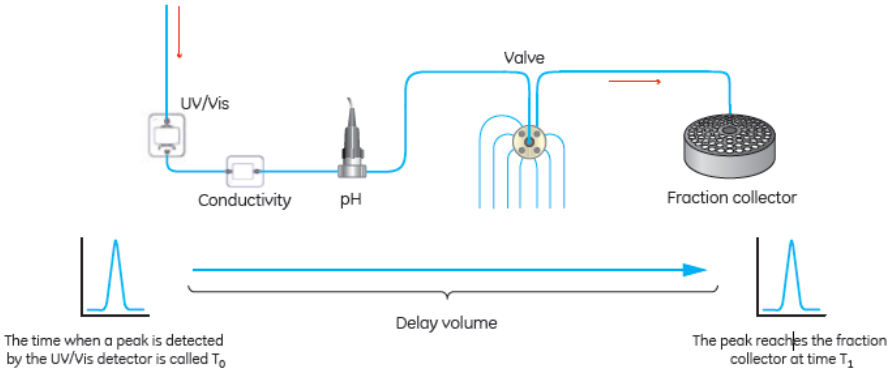
Component	Volume µL
Injection valve **V9M-J**	0.8
UV cell **U9-2M**	2.6
Conductivity monitor **C9M**	5.4

Component	Volume µL
Outlet valve **V9M-Os**	0.7
Flow restrictor FR-902	10
Micro nozzle	1.6

5.5 Delay volumes

Explanation of delay volume

The delay volume is the volume between the detector and the fraction collector or outlet that is used. It is needed to get correspondence between the fractions displayed in UNICORN and the fractions collected in the fraction collector. The illustration below shows and example of the delay volume between the UV monitor and the fraction collector.



Standard delay volumes for the Micro kit

The table below shows the delay volumes with standard configuration of the Micro kit.

Tubing kit	Delay volume Monitor to outlet valve	Delay volume Monitor to frac F9-R
Blue i.d. 0.25 mm	38 µL	60 µL
Red i.d. 0.13 mm	12 µL	18 µL

Theoretical determination of delay volume

To determine the delay volume do the following:

1. Identify all components in the system flow path that contribute to the delay volume of interest.

2. Determine the internal volumes of all hardware modules and tubing, see *Section 5.3 Flow path and tubing, on page 28* and *Section 5.4 Component volumes, on page 29*.

3. To obtain the total delay volume, sum up half of the total cell volume of the UV monitor used with all volumes of tubing and components that are located after the UV monitor in the flow path.

Example to theoretical calculate the delay volume

The table below shows the theoretical examples of the delay volume.

½ UV total cell volume	1.3
Tubing 7	2.2
Conductivity monitor	5.4
Tubing 8+9	2.2
Outlet valve	0.7
Frac tubing	5.1
Micro nozzle	1.6
Sum	**18.4 µL**

Note: *This is rounded to 18 µL in UNICORN.*

Set the delay volume in UNICORN

Step	Action
1	Select ***System:Settings*** in the ***System Control*** module.
2	Select ***Tubing and Delay Volumes***

Step	Action
3	Select ***Delay volume: Monitor to outlet valve***. Type in the volume in the ***Volume*** field and click ***OK***
	Note:
	*The system use the delay volume appropriate to the configuration used and ignore other settings (e.g. the value for **Monitor to outlet valve** is ignored if using a fraction collector). It is however recommended to set all delay volumes so that the volumes remain correct if you change fractionation method.*

5.6 Node IDs

For information on setting or checking Node ID see *ÄKTA pure User Manual 29119969*

Modules in the Micro kit

The table below shows the ID of the modules in the Micro kit.

Module	Node ID
Mixer M9	0
Conductivity monitor C9M	0
Outlet valve V9M-Os	19
Injection valve V9M-J	25

Other modules

The table below shows other modules that are compatible with ÄKTA pure 25.

Module	Node ID
Inlet valve V9-IA	0
Inlet valve V9-IB	1
Inlet valve V9-IAB	3
Inlet valve X1	15
Inlet valve X2	16
Column valve V9-Cs	7

Note: *See User Manual 29119969 for other modules.*

6 Ordering information

The table below shows the accessories and user replaceable spare parts for the Micro kit

Item	Code No.
Micro kit for ÄKTA pure 25	29302910
Injection valve (V9M-J)	29502123
Outlet valve (V9M-Os)	29502129
UV Flow cell U9-2M	29507801
Conductivity Monitor C9M	29298326
Tubing kit, Micro	29261880
Micro nozzle F9-R	29501533
ON-LINE FILTER (10 ML/MIN) CPL	18111801
Mixer chamber 0.6ml	28956186
Flow restrictor FR-902	18112135
Fraction collector F9-R	29011362
I/O-box E9	29011361
Module Panel	29011364
Multidirectional column clamp	29339864
Sample loop 10 µL	18112039
Sample loop 50 µL	29325047
Sample loop 100 µL	18111398
Injection kit	18111089
UV Test Kit, 1 and 2 mm	29276997
Union 1/16" Male - Union 1/16" Male – i.d. 0.25 mm	18112092
Union 1/16" Male - Union 1/16" Male – i.d. 0.13 mm	18112090

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29337720 AC V:1 09/2020