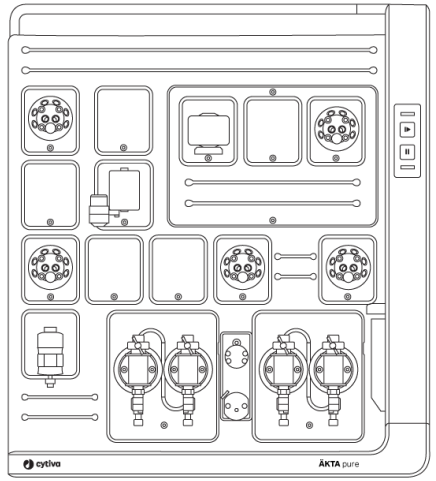
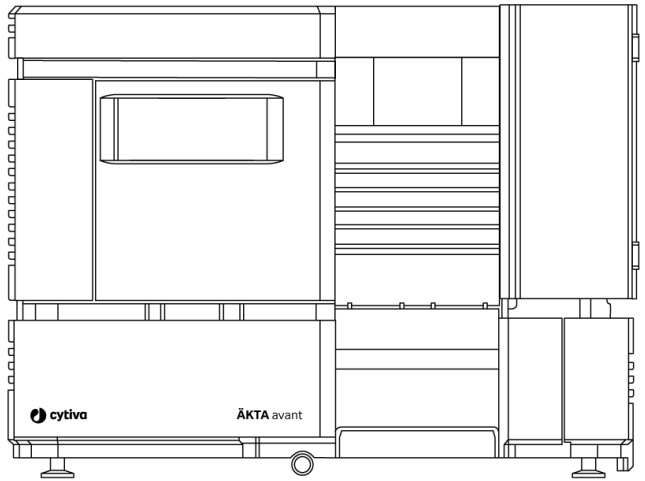
Automated sample application using ÄKTA avant™ or ÄKTA pure™ with an external autosampler

Cue card – autosampler setup and programming





Contents

Cue card - autosampler setup and programming

This cue card describes how to automate protein purification by combining ÄKTA avant™ or ÄKTA pure™ chromatography system with an external autosampler that holds sample volumes in the range of 10 mL – 250 mL in tubes or bottles. It will guide through the installation of the equipment, communication, and method programming.

1

Instrument setup

Teledyne® CETAC® ASX-560 autosampler

The ASX-560 autosampler allows for large volume sample loading and can load more samples compared to the sample inlet valve (V9-IS). The 50 mL racks (Teledyne part number: 450059) hold 84 tubes in total. The 250 mL bottle rack (Teledyne part number: SP7640) holds 25 bottles and the universal bottle rack (Teledyne part number: 32-0748-047) holds 18 or 21 bottles. This section describes the setup and installation of the ASX-560 autosampler. More details about this set-up can be found in the Installation Guide (Teledyne Manual, part number 480237 Rev 1) that is delivered with the instrument.

Installation steps for the Teledyne ASX-560 autosampler

- Mount the Z-drive assembly
- Connect the rinse station and the peristaltic pump
- Install the sample probe
- Assemble the sample vial racks
- Connect the autosampler to the power supply, ÄKTA™ system, I/O box and the host computer

Teledyne ASX-560 Autosampler

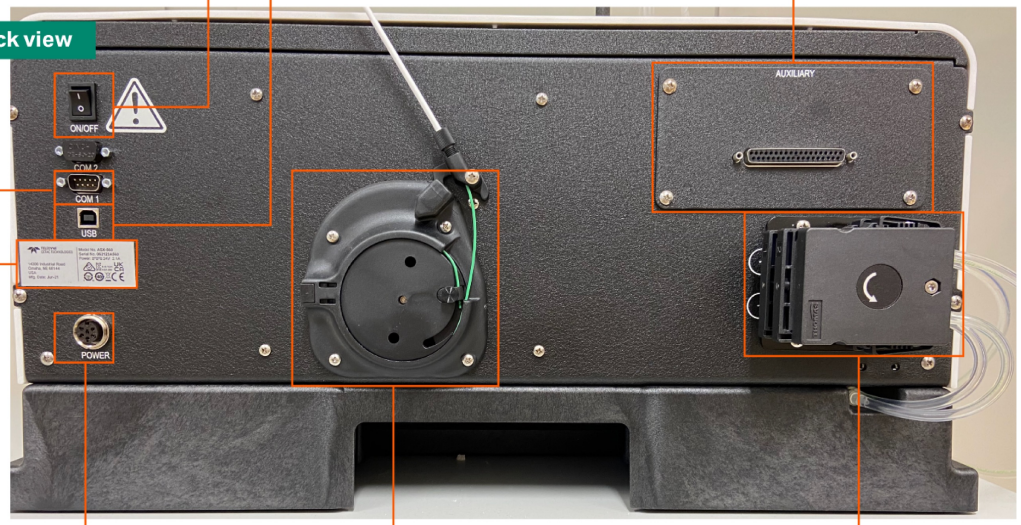
Power Switch

USB Port*: connects the autosampler to the host computer

Auxiliary I/O connector: connects the autosampler to the I/O box

The COM1 port*: connects the autosampler to the host computer

Back view



Info: Serial number. Will be needed for the installation of the software.

Z-drive motor

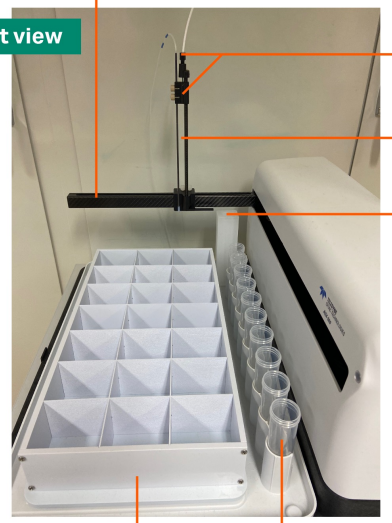
Use either serial or USB connection

Power Connector: The autosampler is powered by the supplied external "brick" power supply

Peristaltic pump: The peristaltic pump moves the rinse agent from the rinse source through the rinse station

Arm

Front view



Z-drive

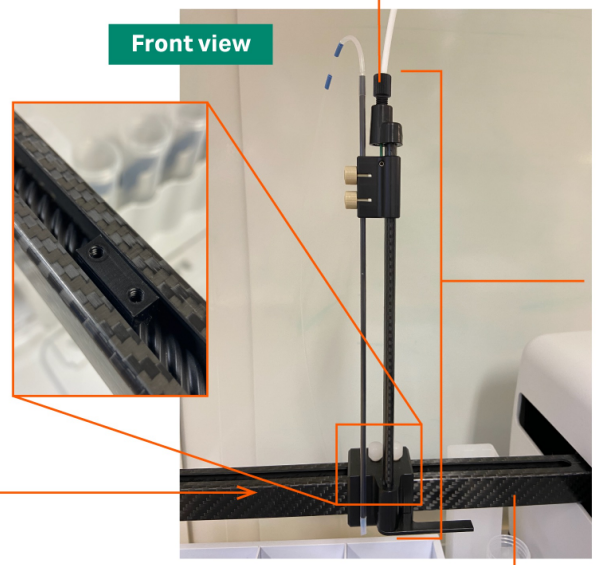
Sample probe

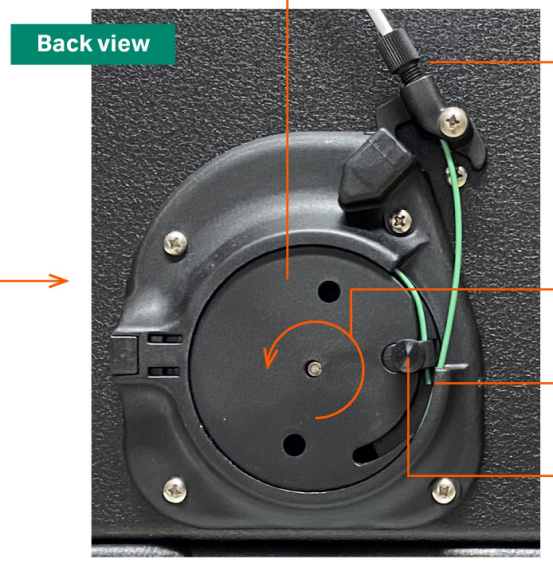
Dual rinse station

Standard vials

Universal bottle rack

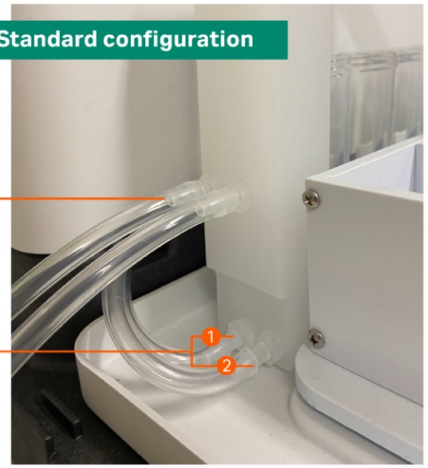
Z-Drive assembly





Dual Rinse Station

Standard configuration

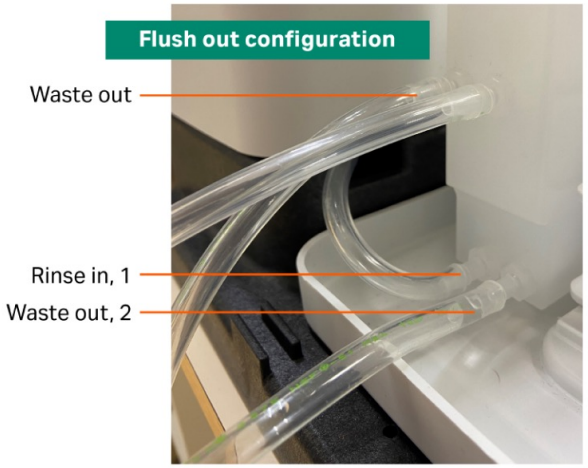


Both position 1 and 2 are connected to the peristaltic pump.

The autosampler peristaltic pump can supply the ***Dual Rinse station*** with two different rinse solutions.

It is also possible to allow the peristaltic pump to supply one rinse solution to position 1 of the dual rinse station, which allows for external cleaning of the probe. Position 2 of the Dual Rinse station can then be used as a pure outlet in the ***Flush out configuration***.

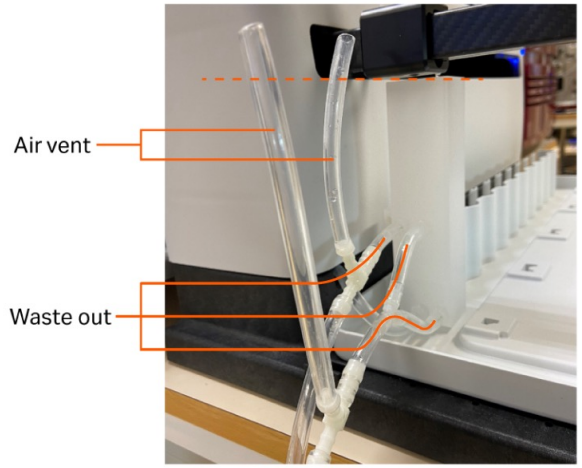
Flush out configuration



Position 1: connected to the peristaltic pump and used for external cleaning of the probe.
Position 2: used as an outlet only.

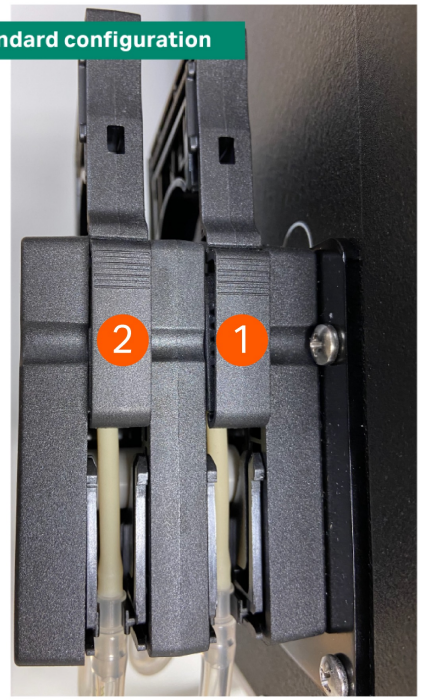
To purge air from the rinse system place the rinse agent uptake tubing in the rinse agent source and run the rinse agent through the rinse station.

The peristaltic pump can be started from IQ-OQ in the ASX Dashboard.

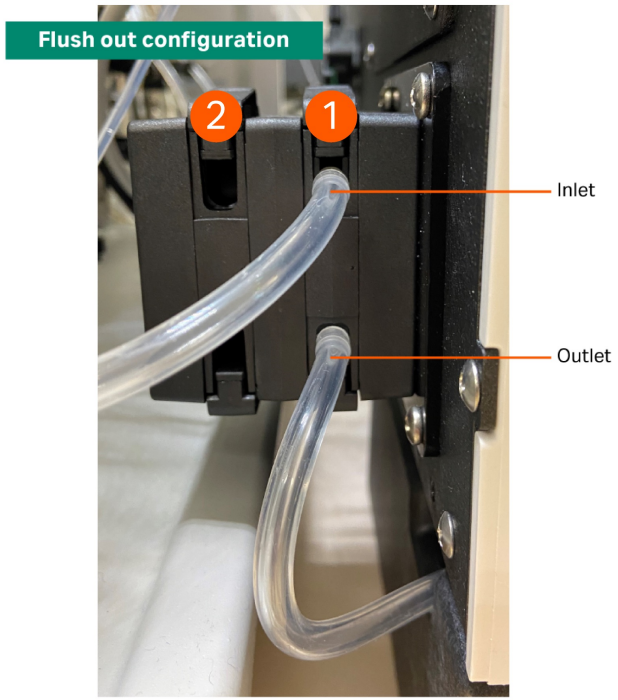


To prevent flooding **air vent tubings** can be added. These have to reach above the maximum liquid level in the rinse station.

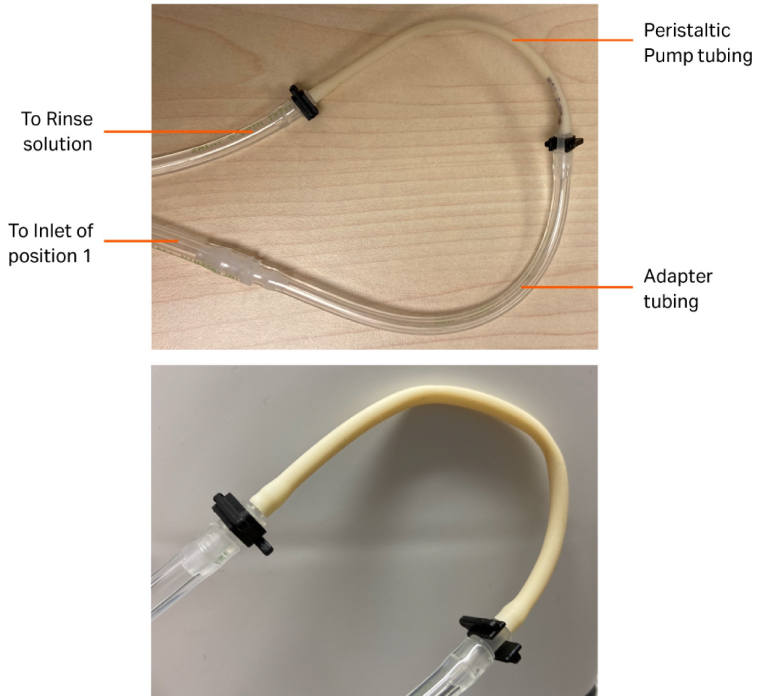
Peristaltic pump



Peristaltic pump 2 mm ID tubing connected to both position 1 and position 2.



Tubing connected into the pump (from the rinse solution) and out from the pump (to the inlet of position 1).



Peristaltic Pump 2 mm ID Tubing (black ends) recommended.

Sample Probe

Shut down the autosampler.

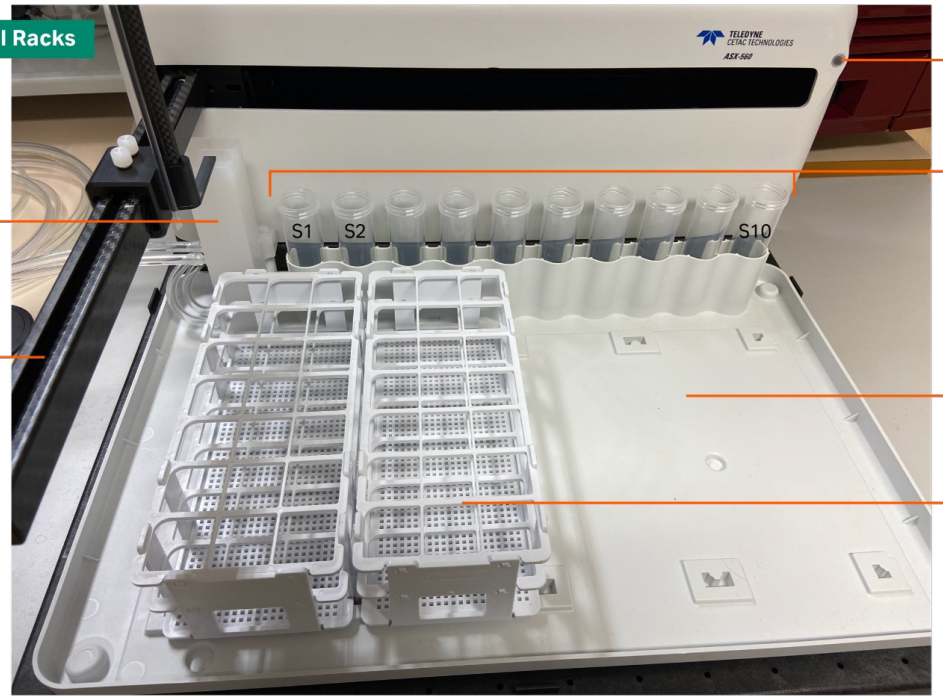
Move the Z-axis slider to the top of the Z-drive by rotating the rotor on the back of the autosampler.

Guide the probe through the two clamps until the probe is level with the bottom of the Z-Drive. Tighten the probe clamps.

Secure the sample tube to the Z-drive cable with the provided spiral wraps. There should be a little curve to the free sample tube to allow to move freely to the corner sample positions and when lowered into the tubes.

Sample vial Racks

Sample vial Racks



Power Indicator Lamp

Inlet

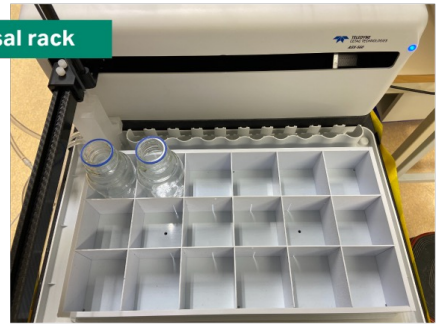
Dual Rinse Station

Arm

Sample Tray

Sample Vial Racks (21-position)

Universal rack



Universal rack (32-0748-047)

250 mL bottle rack

250 mL bottle rack (SP7640)

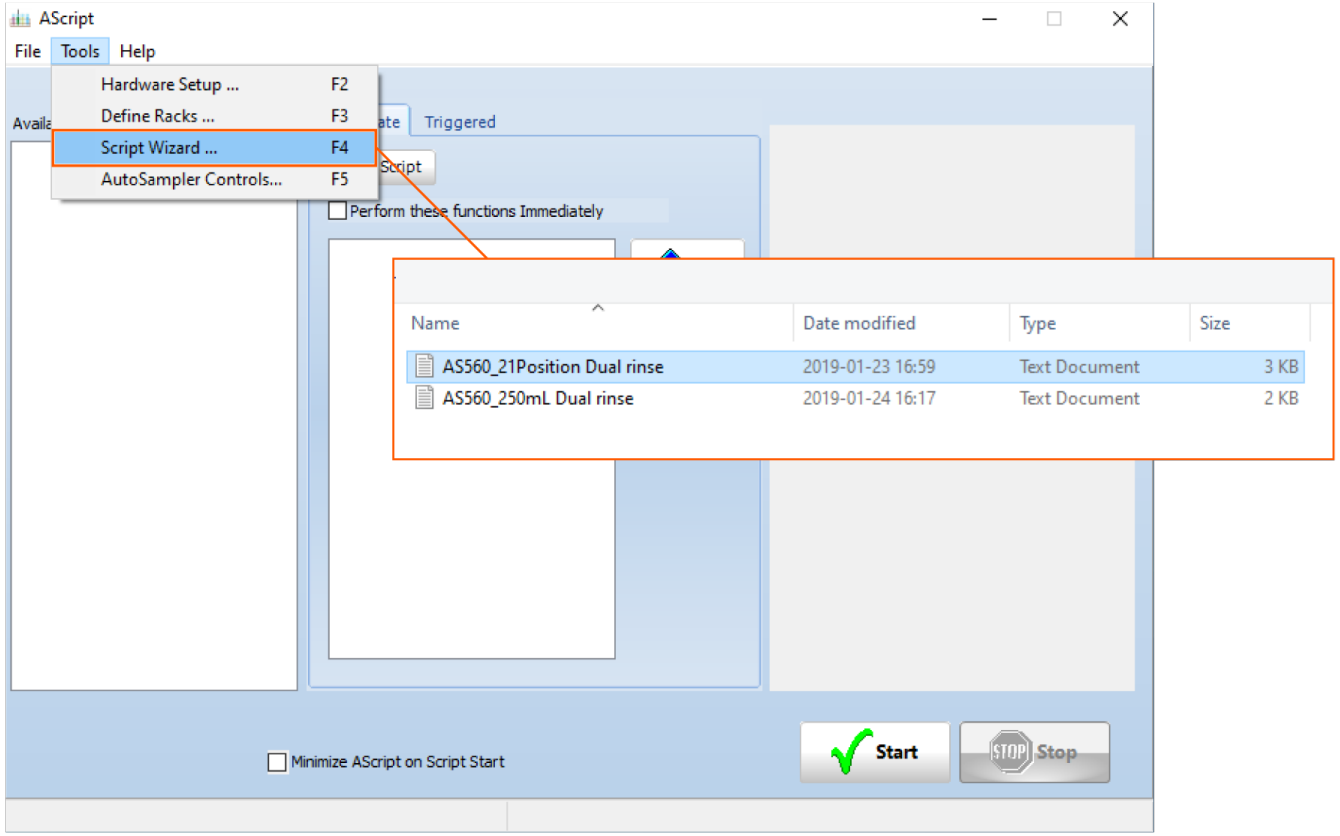
Note. It is important to choose the correct rack definition file in AScript that corresponds with the setup to be used.

Rack definition files

There are different sizes of sample racks and vials that fit the autosampler. **The sample tray is compatible with the 4x21 position 50 mL rack, the universal rack and the 250 mL Bottle rack.** The rack type is defined by **the rack definition file** corresponding to the setup. The file is selected from the **tools tab in the AScript software**.

Note: The sample positions are defined in the instrument control software, be sure to use the correct racks.

Note: When using the Dual rinse station, the coordinates for position 2 in the Dual rinse station are written in the position for the standard vial 1 (S1), hence S1 cannot be used as a standard vial.



ÄKTA™ chromatography system

Both ÄKTA pure™ and ÄKTA avant™ can be connected to the ASX-560 autosampler. Key modules for connecting and operating the autosampler are a **sample pump (P9-S)**, **sample inlet valve (V9-IS)** and an E9 **I/O box**. This instruction also includes extensions to this standard setup, allowing for a more efficient cleaning of the sample probe (Flush out configuration).

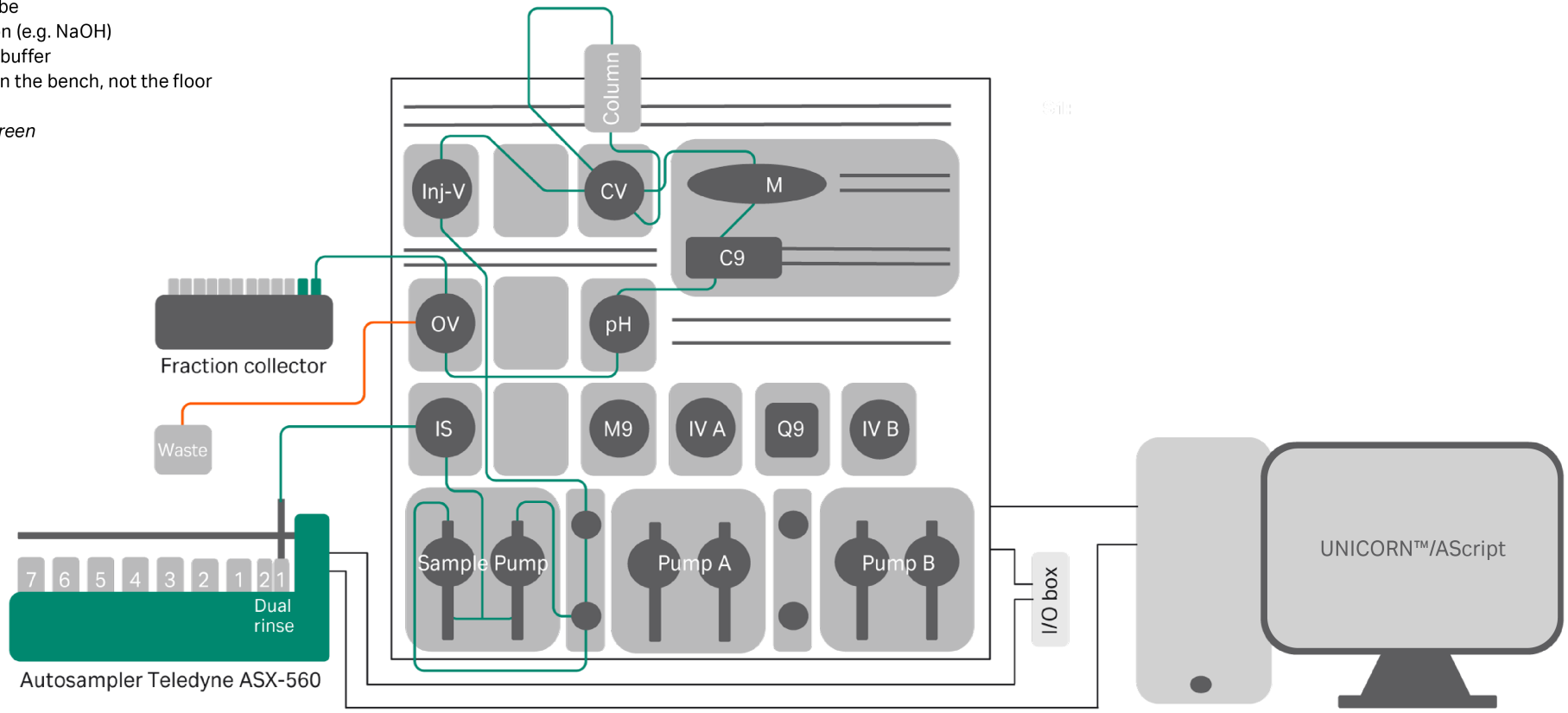
Standard configuration

In the *standard configuration* the sample is loaded from the autosampler via the probe and the S1 inlet on to the connected column. Sample application can be performed with a fixed volume or until air is detected in the air sensor. In this configuration two different solutions can be used to clean the probe.

Standard configuration

S1: Connected to the sample probe
Rinse station 1: cleaning solution (e.g. NaOH)
Rinse station 2: sample loading buffer
Rinse solutions bottles must be on the bench, not the floor

Sample flowpath is displayed in green

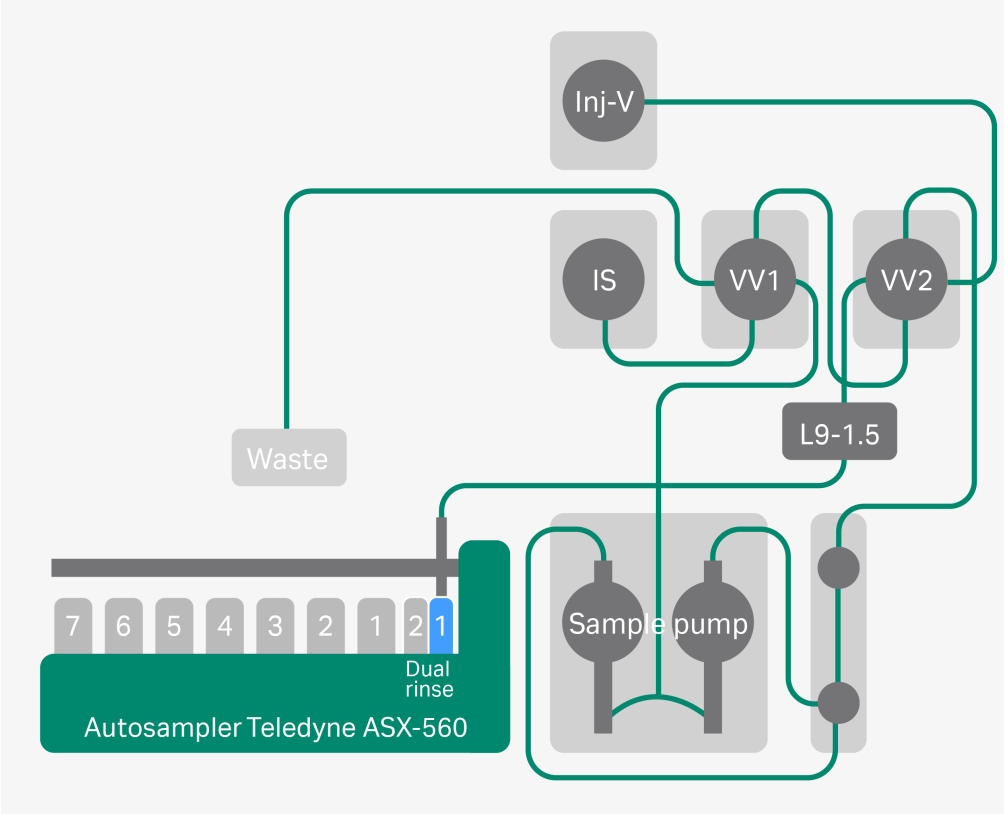


Flush out configuration

- The *Flush out configuration* is recommended for a more robust and reliable cleaning of the sample probe. In the *Flush out configuration* the autosampler probe is washed using the ÄKTA™ system by selecting rinse solution from the sample inlet valve and reversing the flow out through the probe into position 2 of the dual rinse station. In addition to the modules required in the standard configuration **two versatile valves (V9-V)** and **one external air sensor (L9-1.5 mm**) are required. The dual rinse station setup and use is different from the standard configuration.
- **Advantages with using the Flush out configuration:**
- Possible to change/include **more than one rinse solution** for cleaning of the inside of the probe (select different sample inlets).
- More **flexibility in** rinse solution **volume** and **flow rate** (programmed in the UNICORN™ method)
- The maximum sample pump flow rate that can be used without flooding the rinse station, depends on the tubing dimensions connected to the dual rinse station and the speed of the peristaltic pump as well as the ÄKTA™ system setup.
- If running a method without the autosampler, using the flush out configuration, the versatile valves have to be in the following position: versatile valve 1: 1-4, 2-3, versatile valve 2: 1-2, 3-4. It is possible to change the default positions in Systems settings for the versatile valves (ÄKTA™ pure). If not possible to change in systems settings include these instructions in the beginning of the method.

Flush out configuration

Flush out configuration setup with key modules and capillary connections included (displayed in green). This setup requires two versatile valves (V9-V) and one external air sensor (L9-1.5)



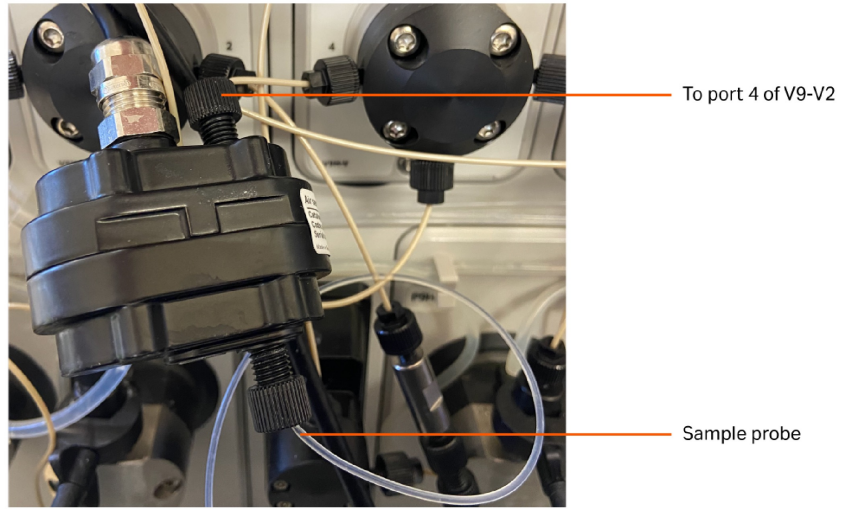
Connect the Sample probe of the Autosampler to the ÄKTA™ chromatography system

Determine the length of the sample transfer tubing (attached to the probe) and connect it to the ÄKTA™ chromatography system.

Use a ferrule for 1/16″ tubing and a tubing connector 1/16″ M to connect the sample probe to the ÄKTA™ system.

In a ***standard configuration***, connect the tubing from the sample probe to port S1 of the sample inlet valve (V9-IS).

In the ***flush out configuration***, connect the tubing from the sample probe to the external air sensor. Connect the air sensor to port 4 of the second versatile valve (V9-V2).



2

Communication

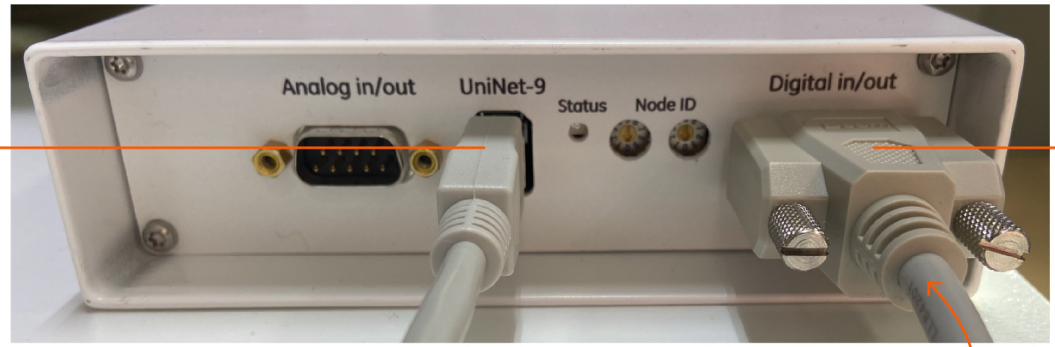
Cable connections

The autosampler is powered by the included external power supply.

It is possible to use either USB or serial connection between the ASX-560 autosampler and the host computer. When using the ASX dashboard, we recommend USB.

Connect the USB or serial cable between the autosampler and the host computer. If using a serial cable connect it between a serial port on the host computer and the COM1 port on the autosampler.

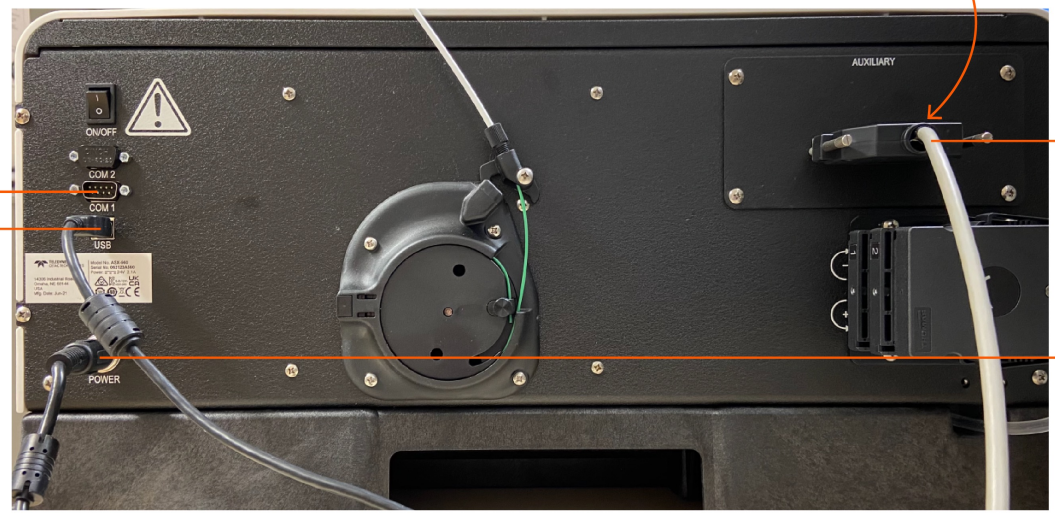
UniNet-9: Connects the I/O box to the host computer



Connect **the 9-pin end** of the **custom cable** to the Digital In/Out port of the ÄKTA pure™ I/O box

COM1 port: connects the autosampler to the host computer via serial connection

USB port*: connects the autosampler to the host computer



Connect the **37-pin end** of the **custom cable** to the ASX 560 I/O Board

Power Connector: The autosampler is powered by the supplied external "brick" power supply

* recommended

Custom I/O cable

To be able to communicate and synchronize events between the ÄKTA™ chromatography system and the external autosampler a I/O card must be installed on the autosampler. Through the UNICORN™ software and the AScript software it is possible to send and receive Digital signals which can control e.g., the movement of the probe and allow for sample application.

Connect the 37-pin end of the custom cable, supplied with the ASX-560 autosampler to the Auxiliary port on the autosampler and the 9-pin end of the custom cable to the Digital In/Out on the E9 I/O box.

3

Teledyne Software

Software and documentation on the CD:s included with the Teledyne ASX-560 autosampler

AScript CD for ÄKTA™ *(CD Part Number 670138)*

- **AScript** – AScript Setup 1.5.1
- **Dual rinse racks** – Dual rinse racks (text files) and Script* for AScript with dual rinse station.
- **ÄKTA™ Scripts** – 50mL ÄKTA™ script smaller rinse*
- **AScript Config Guide and ASX-560 ASX-280 Quick Installation Guide for ÄKTA pure™**

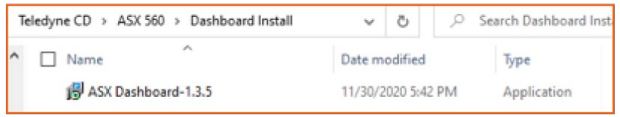
* *The cue card contains updated scripts for both the Standard configuration and the Flush out configuration.*

ASX 560 *(CD Part Number 670130)*

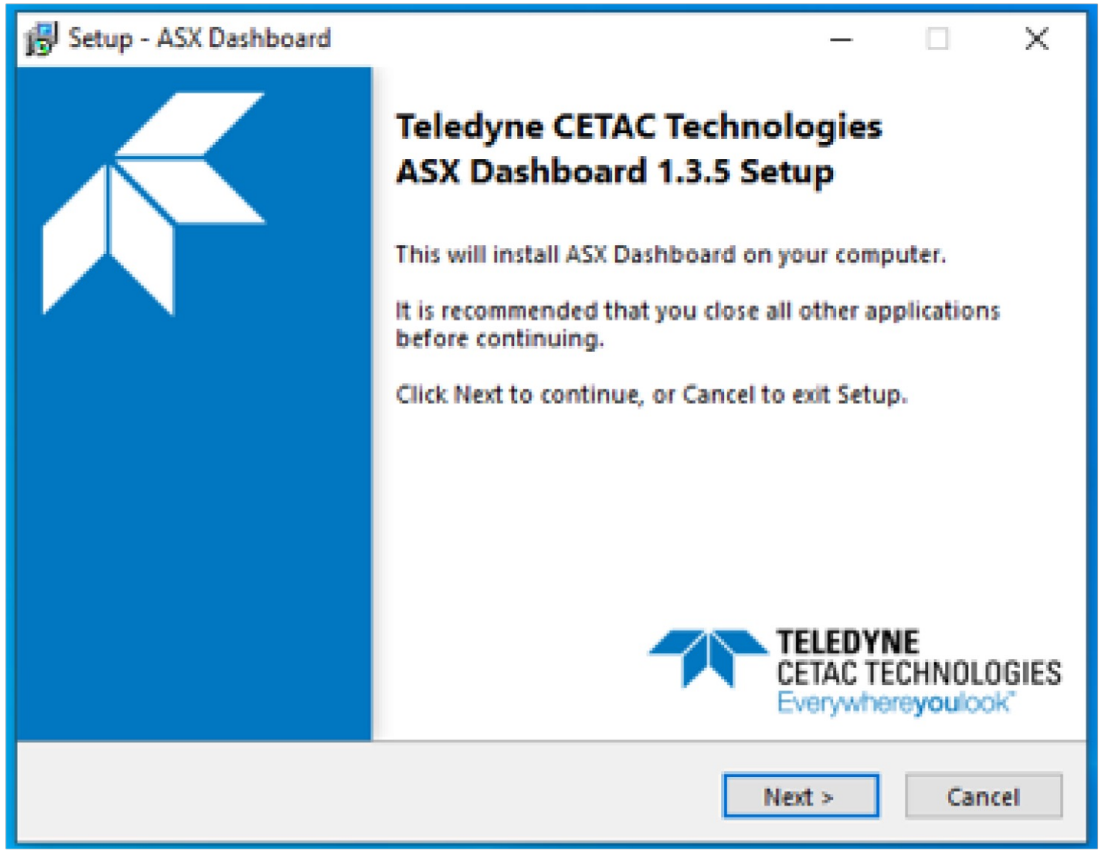
- **Alignment Documents** - Alignment Files and Guide
- **Dashboard Install** – ASX Dashboard- 1.3.5
- **Rack Files** – N/A
- **USB Driver** – CDM20824_Setup
- **ASX-560 ASX-280 Operators Manual, ASX-560 ASX-280 Quick Installation Guide, ASX-560 Series Autosamplers Safety Manual, Waste Electrical and Electronic Equipment**

Install the ASX Dashboard

To **install** the **ASX Dashboard** software double click on the installation file which can be found on the CD.



Follow the prompts to complete installation. A USB driver is included in the installation.



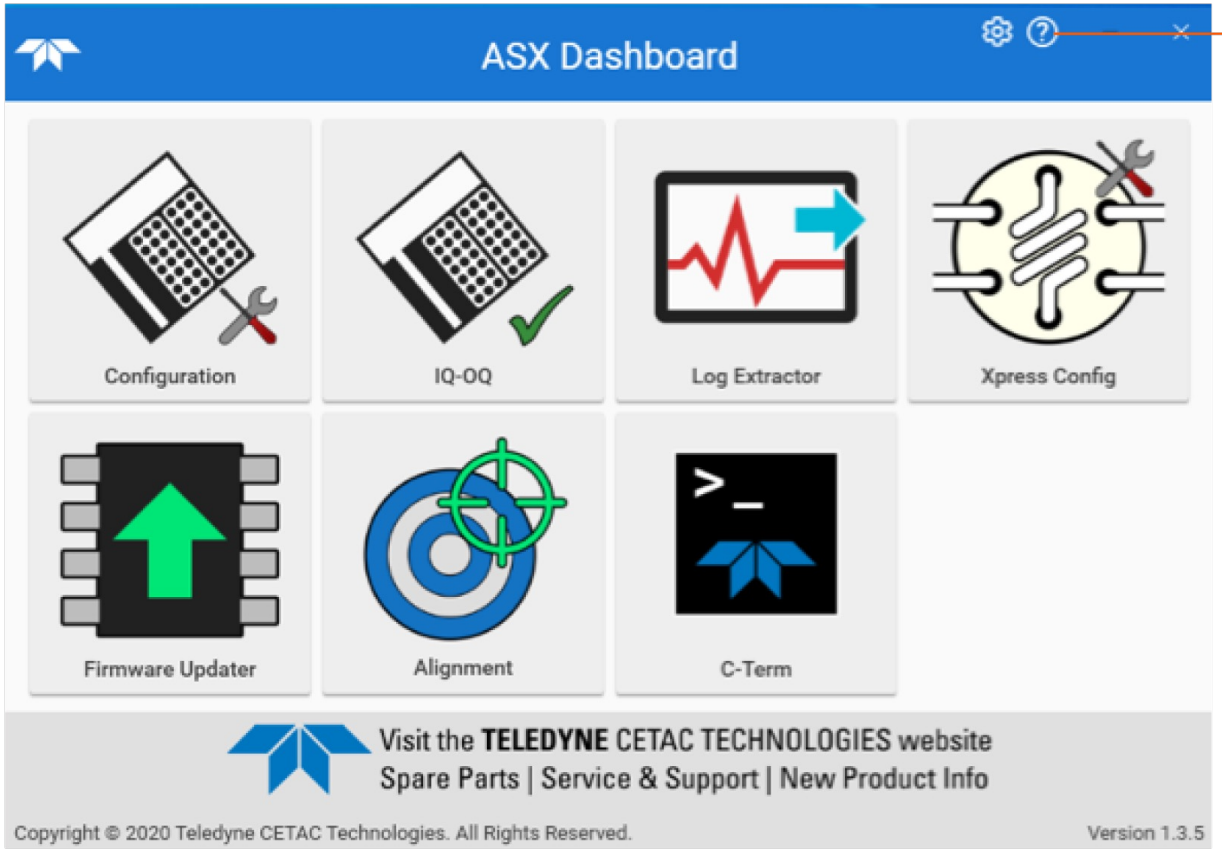
The ASX Dashboard Interface

Configuration

- Set the speed of the vertical motion of the sample probe, **Z/W Speed**
- Set the Peristaltic **Pump Speed**
- **Restore default** values

Alignment

- Alignment of the sample probe can be performed if it is not centered over the rinse station or sample vials



Help

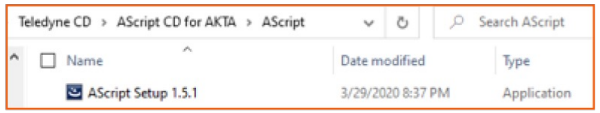
- Help information about each individual utility in the ASX Dashboard

IQ-OQ

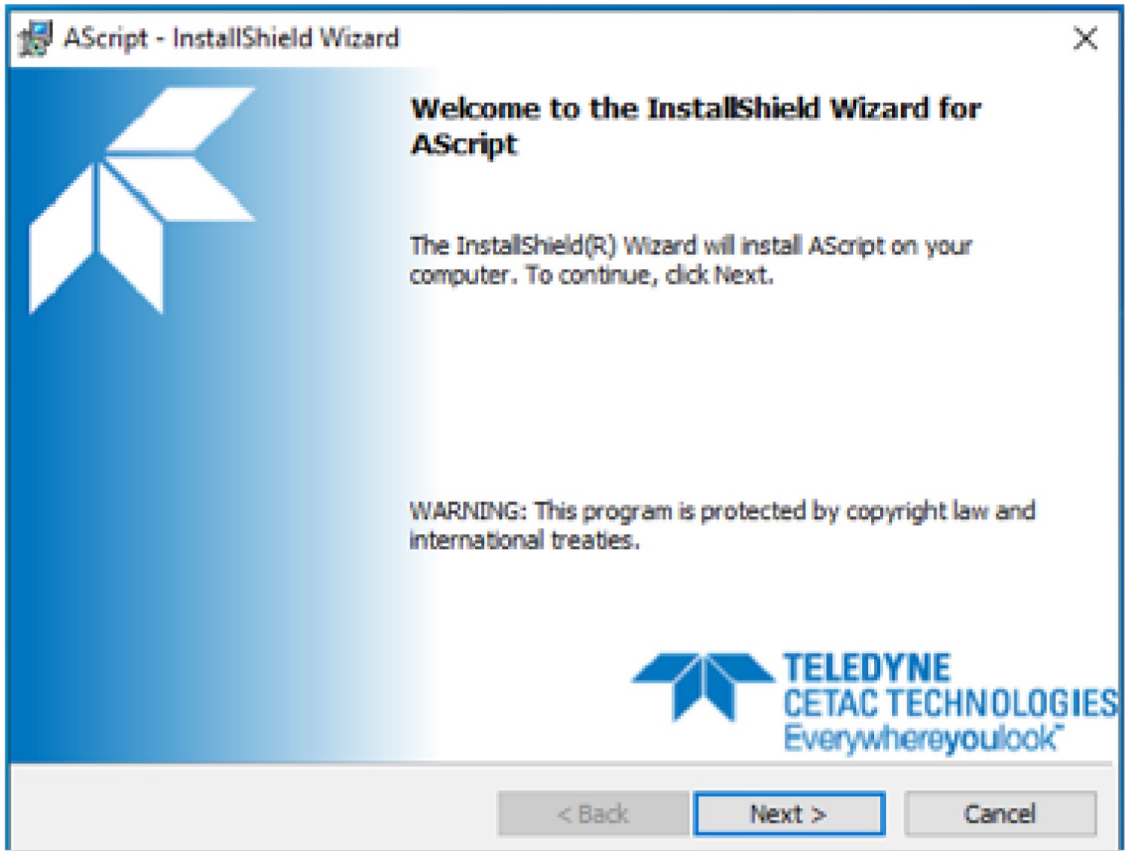
- Perform simple test to verify that the autosampler is functioning
- Control the Auxiliary I/O port
- X-Y-Z movement speed including peristaltic pump speed are relative values which can be adjusted

Install the AScript

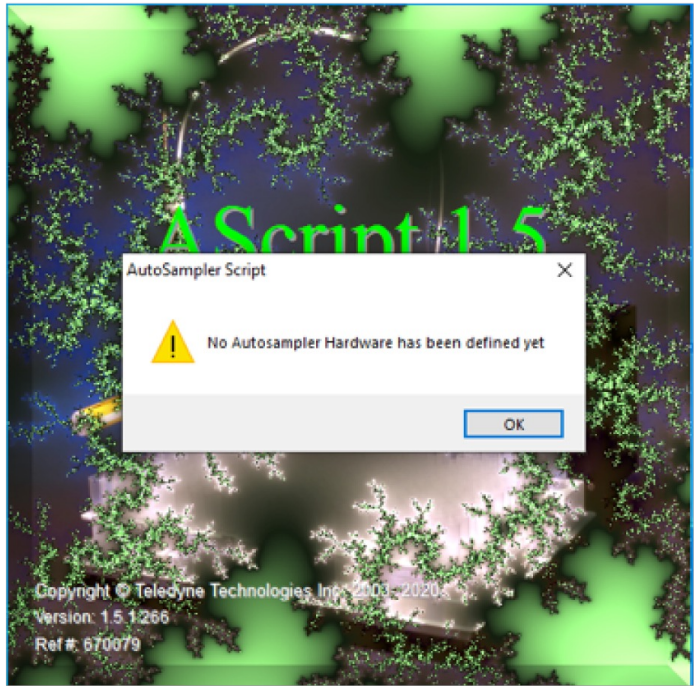
To **install** the **AScript** software double click on the installation file which can be found on the CD.



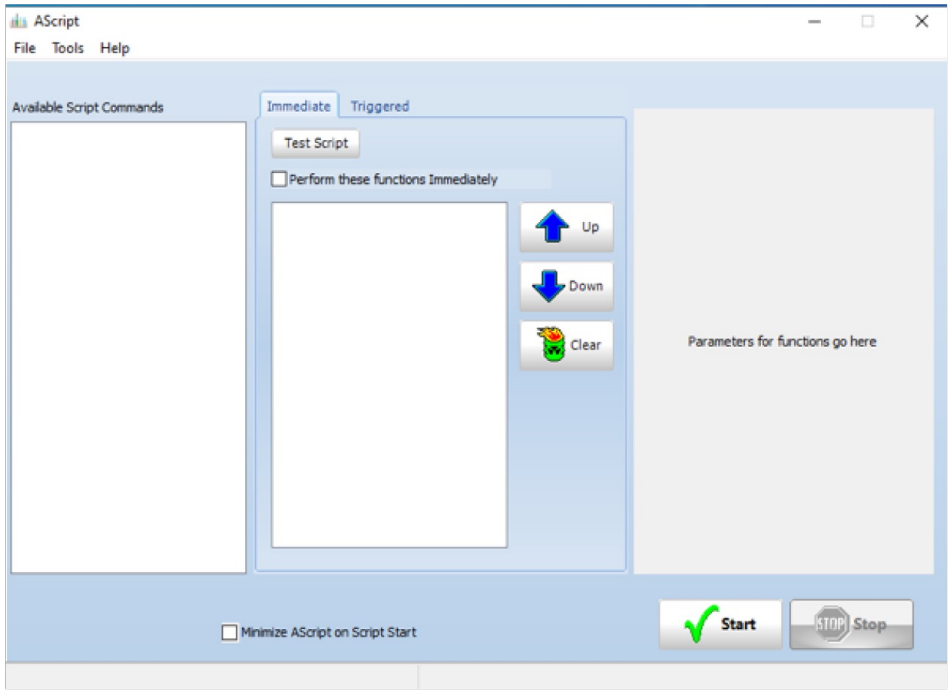
Follow the prompts to complete installation.



Open AScript



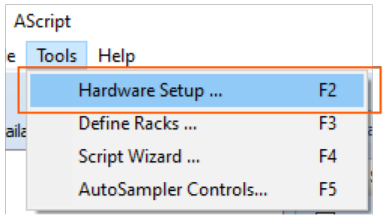
Click **OK** if the warning is displayed



AScript will open

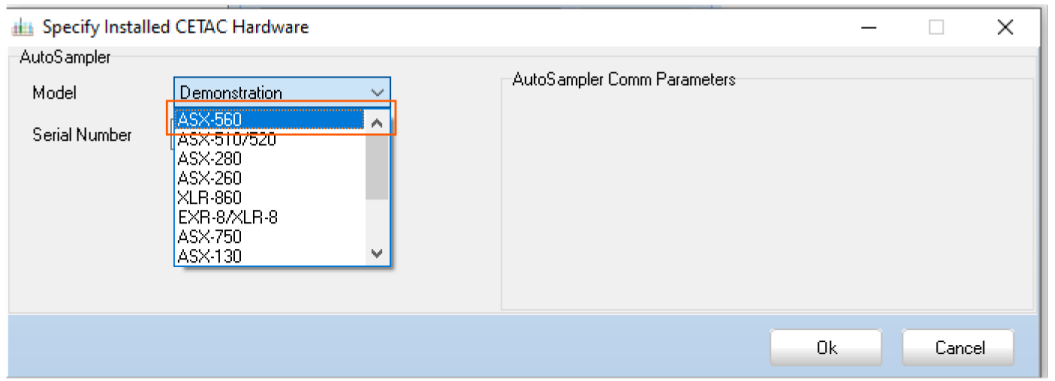
Hardware Setup

Open **Tools** and select **Hardware Setup**

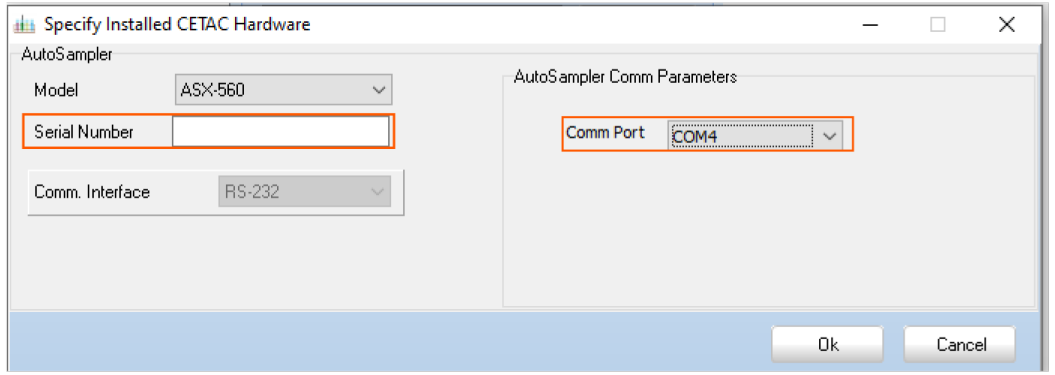


Enter the **Serial Number** (can be found on the backside of the autosampler)

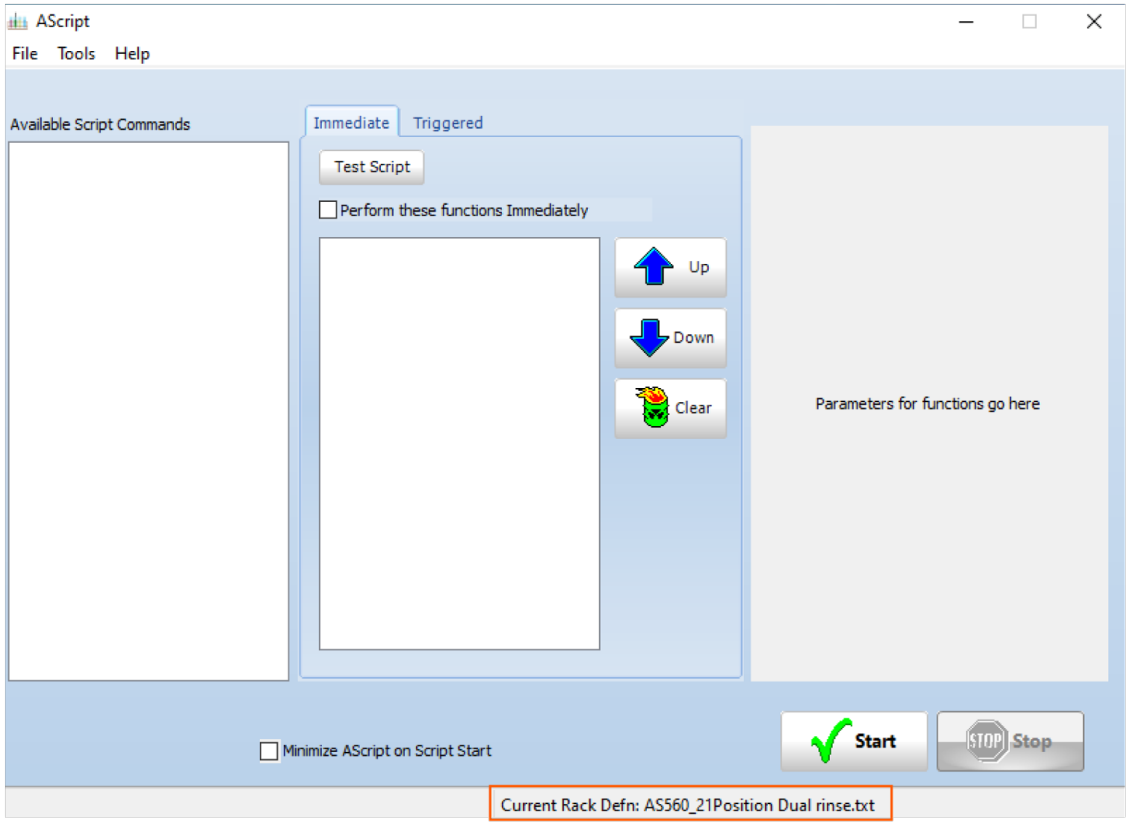
Comm Port: COM1-3 are **serial connections**. COM4 and above are **USB connections**. If using a USB connection, a COM Port will be assigned by the USB driver software when the autosampler is plugged in.



Select the **Model ASX-560**

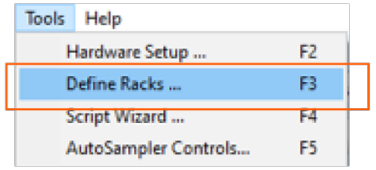


Define racks

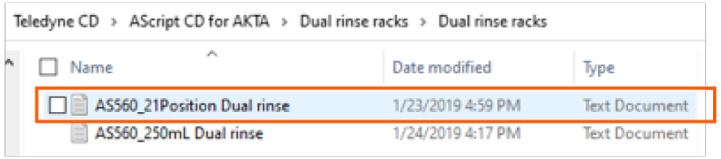


Current defined rack is displayed

Open **Tools** and select **Define Racks**



Select the file with the autosampler, rack number and type of rinse station to be used.



Note: The rack type must be redefined if the type of racks is changed.

Note: Please check that the selected rack file correspond with the rack layout and the bottles to be used before staring a run. This can be tested in AScript by moving the probe to different positions and lower/raise the sample probe.

Caution: perform this operation with empty rack stands.

4

Settings and initial tests

Settings and initial tests

In **UNICORN™** select **Administration - System Properties – Edit** and enable the E9 I/O box. The E9 I/O box enables digital signals to be sent and received between UNICORN™ and the AScript software.

In the *Flush out configuration* also enable the following modules: Versatile valve, Versatile valve 2 and External air sensor (Before sample inlet).

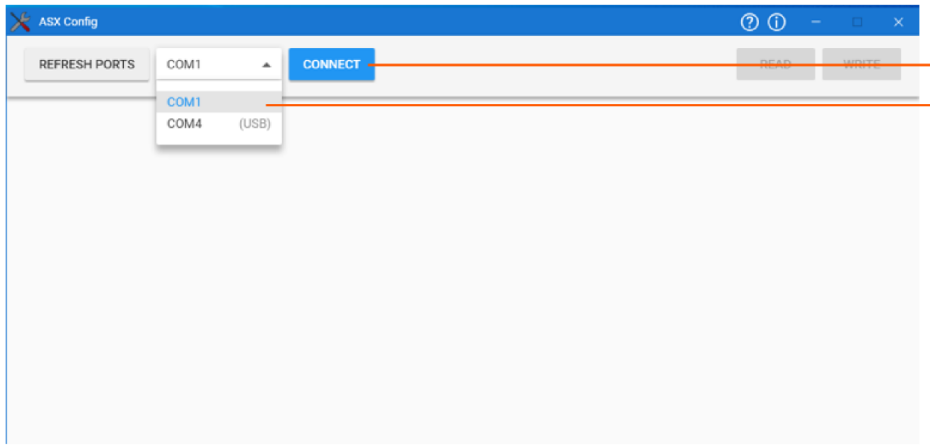
Connect to the ÄKTA™ system, open **System control** and **System settings**.

- Select the **E9 I/O box** and change the values for the **Digital Out signals** from **1 to 0** for all four Digital out signals.
- Select **Advanced** and the **System and pump wash settings**. Change the sample pump speed and pump wash volume to appropriate values. In the *standard configuration* the maximum sample pump speed cannot be higher than the speed of the peristaltic pump, this is to prevent draining the rinse station and introduce air into the ÄKTA™ flow path.

Note: The sample pump speed depends on the system configuration and the tubing dimensions. This must be evaluated for each system setup. As a reference (to prevent flooding the rinse station): peristaltic pump 2 mm ID tubing (black), speed of 100 % is compatible with a sample pump speed of 50 mL/min in the standard configuration for ÄKTA avant™ 150.

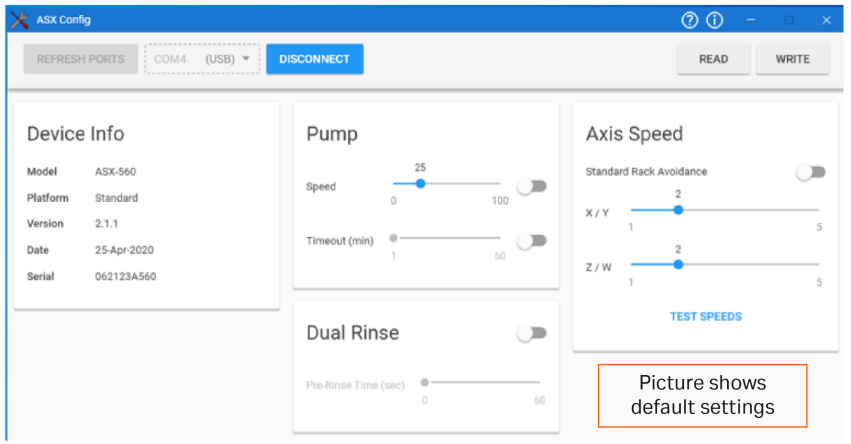
ASX Dashboard

In **ASX Dashboard** open **Configuration**



Press **Connect**

Connect to the autosampler by selecting the **COM port** which corresponds to the **USB connection**, in this case COM4.



Device info is read by the software. *Serial* can also be found on the backside of the autosampler.

* In the *standard configuration* the peristaltic pump supplies the rinse station with liquid used by the ÄKTA™ sample pump in the pump wash. In the *flush out configuration* the peristaltic pump supplies the rinse station position 1 with buffer to rinse the outside of the probe.

Pump: Set the pump **speed** to **100 %** for 2 mm ID tubing in the standard configuration. In the flush out configuration the pump speed can be lower*.

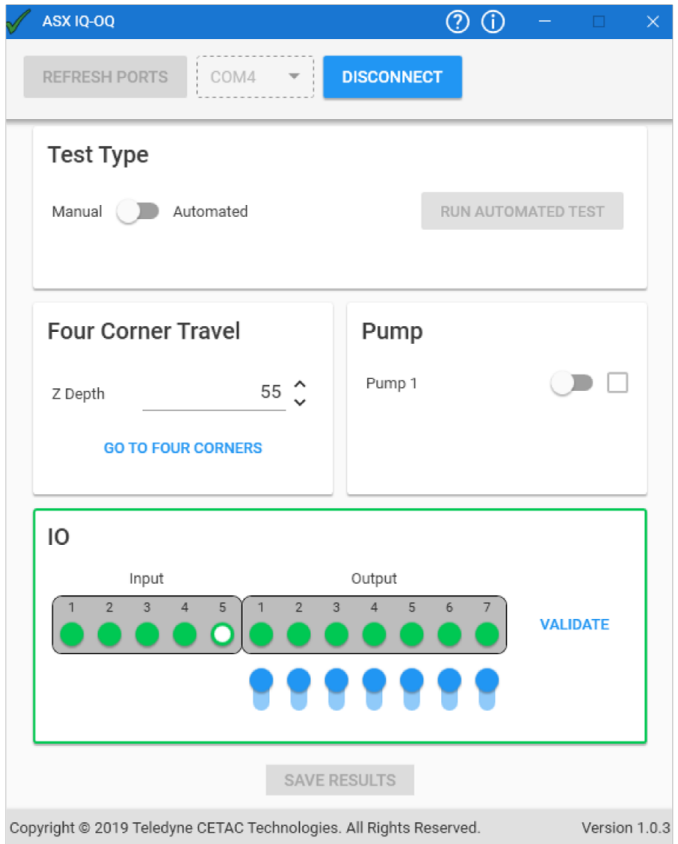
Axis Speed: Change the X/Y and Z speed from 2 to 4.

Click **Write** to update the firmware

Note: The Dual Rinse is Disabled as default and remains as is.

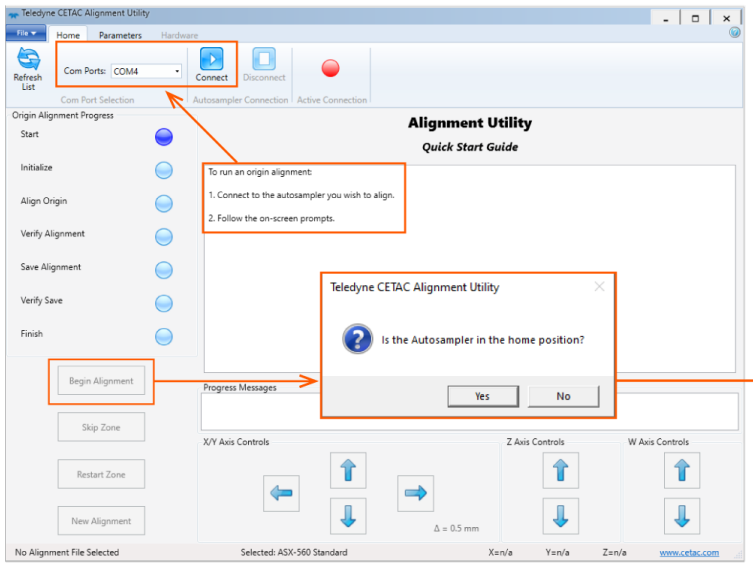
In **ASX Dashboard** open **IQ-OQ**

- Change to **Test Type Manual.**
- **Click Pump On** will fill the rinse line and rinse station with rinse solution.
- **IO:** flip all the **Outputs** to **high** and click **Validate**. It should look like in the figure.

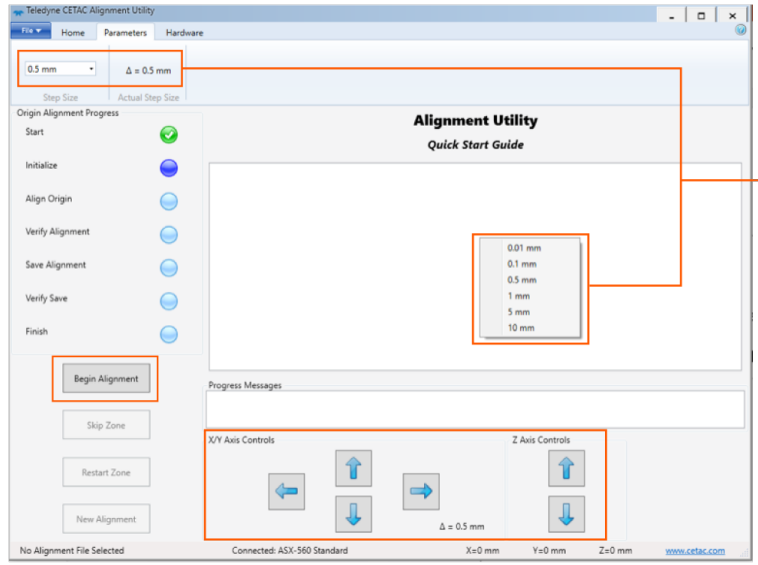


If the sample probe is not centered when in the home position (centered in position 1 of the rinse station), an alignment of the sample probe can be performed in the **ASX Dashboard**.

In **ASX Dashboard** open **Alignment**



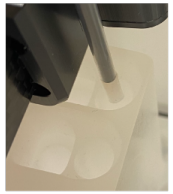
If the autosampler is not in the home position, click No. Cycle power then reestablish communication with the alignment software.

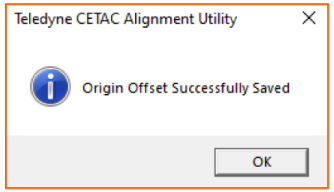


The step size can be changed in **Parameters** or by *right clicking*

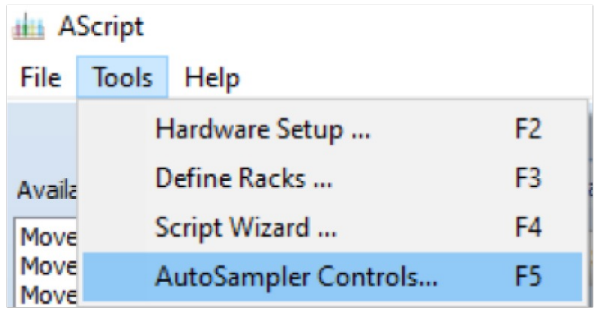
Use the **X/Y Axis Controls** to center the probe tip in the rinse station. The edges of the probe should be equally spaced from the walls of the rinse station (X and Y axes).

Position the probe with the tip approximately 1 cm into the rinse station using the **Z Axis Controls**.

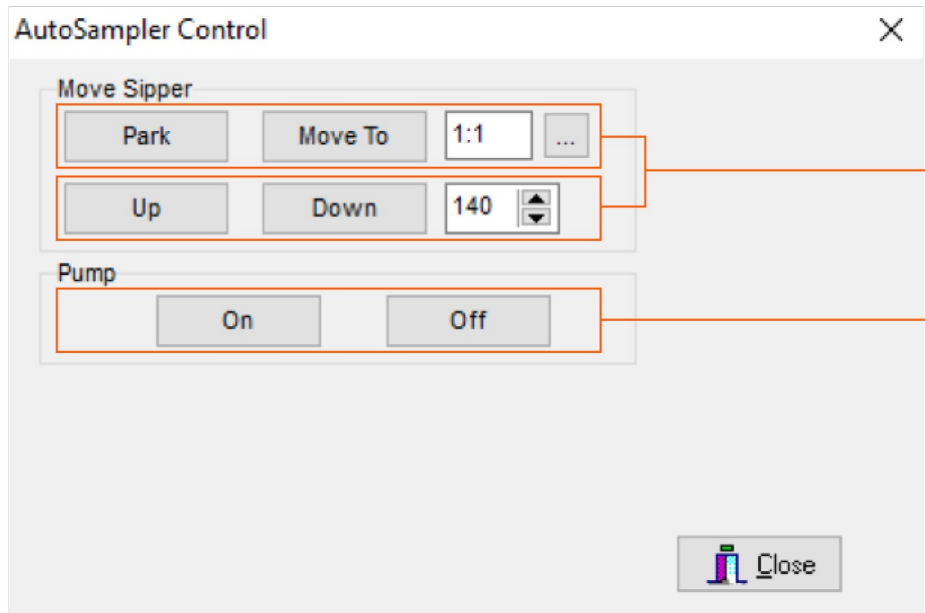




In **AScript** it is possible to test the movement and communication between the autosampler and the host computer. Select Tools tab and **AutoSampler Controls** to test movement to a specific vial.



Open **AutoSampler Controls**: the probe moves to the rinse station and is lowered into position 1. The peristaltic pump is started.



Up/ Down: test the movement of the probe in the Z-direction. Select the depth.

On/ Off: Turns the peristaltic pump on or off.

Park: moves the probe to the rinse station and lowers the probe into position 1 and starts the peristaltic pump.

Move To: test the movement of the probe in the X/Y direction. Select a tube position.

5

Method programming

Method programming

This section explains how to create UNICORN™ and AScript compatible methods.
It includes methods for the following configurations:

- Standard configuration
- Flush out configuration

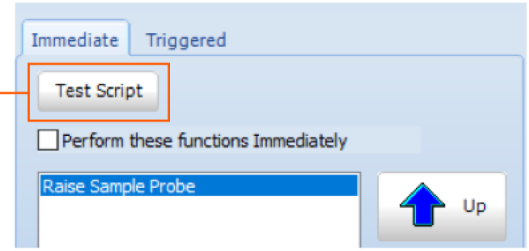
Method programming

The following **AScript commands** are used to move the probe to the Dual Rinse station. Note that the Dual Rinse option should be **disabled** in ASX Dashboard and a **rack definition file** compatible with the dual rinse station should be used.

- Move to Rinse
The probe moves to the rinse station and lowers the probe into **position 1**.
The peristaltic pump is turned ON during the rinse procedure.

- Move to Standard 1
The probe moves to the rinse station **position 2**. It does not lower the probe and the pump is not turned ON.

If the probe moves to the rinse station and performs its initializing routine, the command **Raise Sample Probe** can be executed as an **Immediate** script to turn off the pump and raise the probe from the rinse station. Press **Test Script**.



Digital signals

To allow for communication and synchronization between the UNICORN™ method and the AScript method it is necessary to use digital signals and watch instructions. The methods are programmed to send continuous digital signals from AScript and digital pulses from UNICORN™. There are four ports available for output signals and four ports available for input signals.

Corresponding commands in UNICORN™ and AScript.

UNICORN™		AScript
Digital in	←	Output on Port
Digital out	→	Wait for Port Input

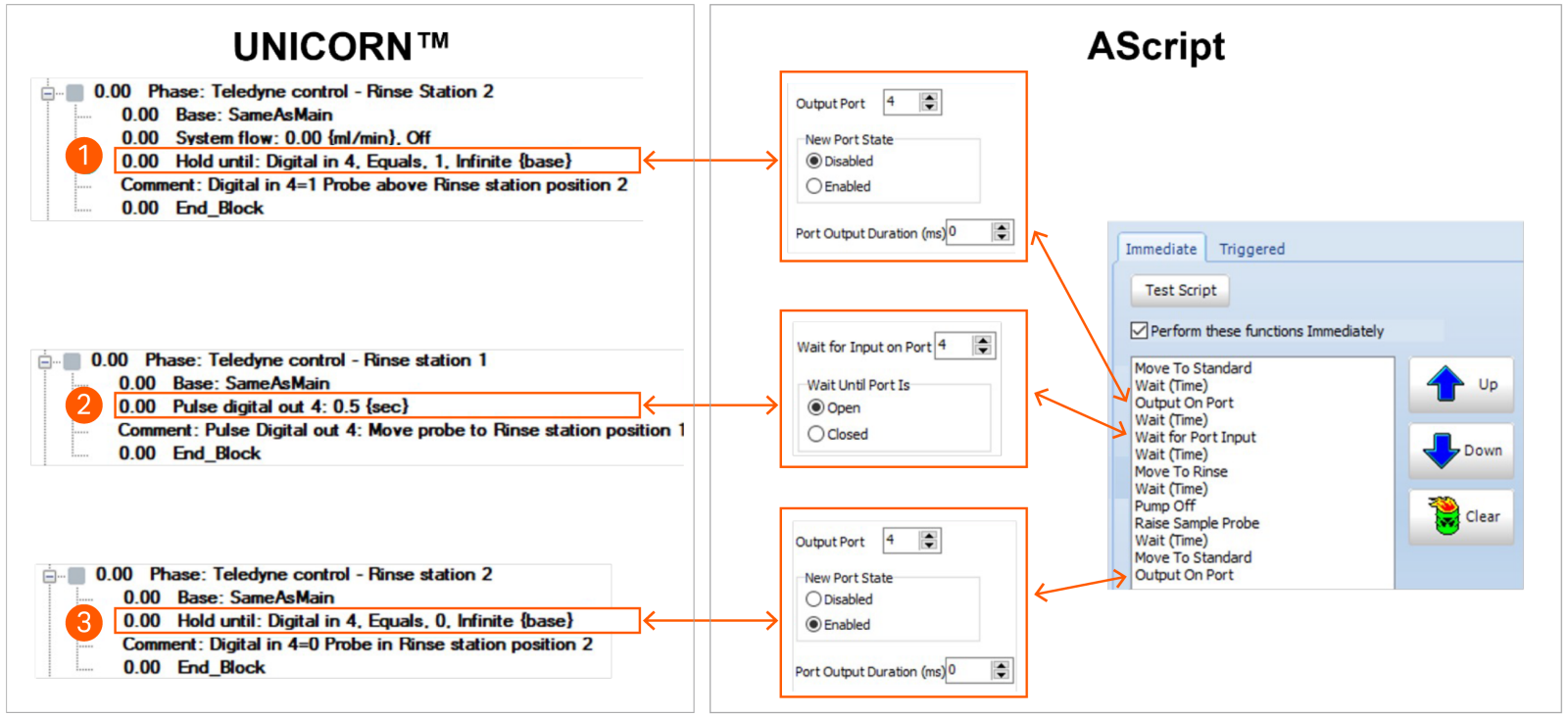
In **UNICORN™**: before starting a run the values for all four **Digital out** signals in must be changed from 1 to 0 in **Systems settings**. This will allow for sending **Digital pulses** from UNICORN™.

In **AScript**: make sure to enable **Output on port** (for signals 1-4) before starting a run, this will put all **Digital in** signals in UNICORN™ to 0.

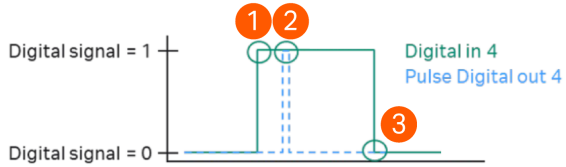
If the Digital in and Digital out signals have not been set to 0 before starting a run the UNICORN™ and AScript methods will not be synchronized.

Note: The measured digital signals can be shown as a curve in UNICORN™. An open versus a closed circuit is interpreted differently in these two software.

Communication between UNICORN™ and AScript



All digital signals are set to 0 in the beginning of the run.



	UNICORN	AScript	
Open circuit	Logical 1	Logical 0	Enabled
Closed circuit	Logical 0	Logical 1	Disabled

UNICORN™ software methods

In the UNICORN™ method, make sure to adjust the sample flow, pressure alarms and wash volumes to suitable values depending on the ÄKTA™ chromatography system and column to be used.

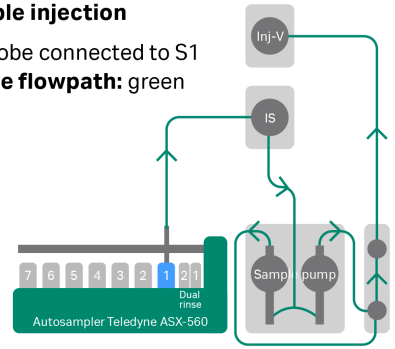
Standard configuration – Sample application and CIP of the probe

The sample probe is connected to S1.

```
0.00  Phase: Teledyne control - Next sample
    0.00  Base: Time, Any
    0.00  Hold until: Digital in 1, Equals, 1, Infinite (base)
    Comment: Digital in 1=1 Teledyne ready for next sample
    0.00  Pulse digital out 1: 0.5 (sec)
    Comment: Move Probe to Next sample
    0.10  Hold until: Digital in 1, Equals, 0, Infinite (base)
    Comment: Probe in Next Sample Tube
    0.10  End_Block
0.00  Phase: Sample Application
0.00  Phase: Teledyne control - Sample finished
    0.00  Base: Time, Any
    0.00  Pulse digital out 2: 0.5 (sec)
    Comment: Sample application finished
    0.00  End_Block
```

Sample injection

IS: Probe connected to S1
Active flowpath: green



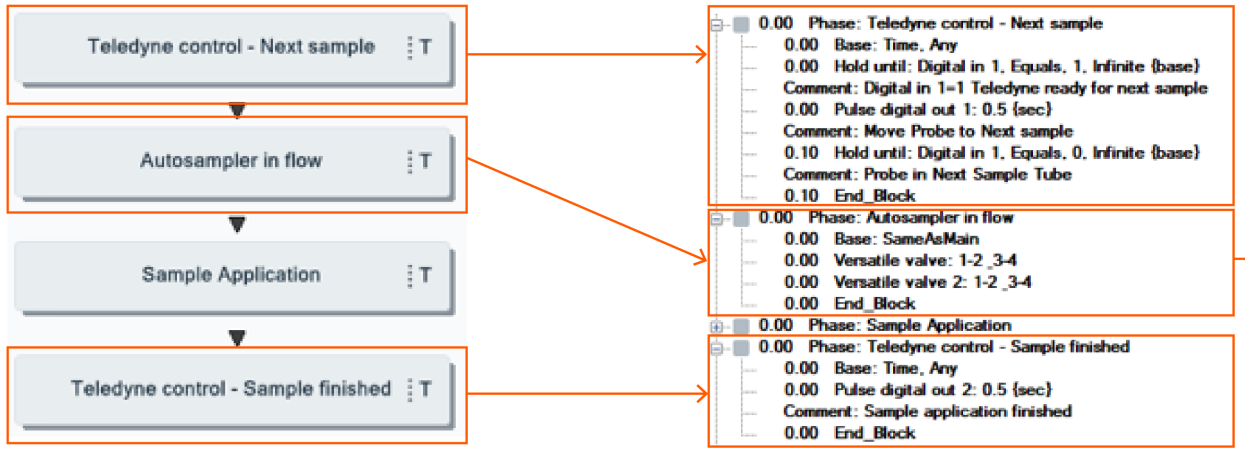
During the phases **Wash Probe** a Sample pump wash is executed and the rinse solution is supplied from the Dual Rinse station position 1 and position 2 by the peristaltic pump.

The UNICORN™ method includes *Teledyne control phases* which can be created from **user defined phases** by adding the **text instructions** as listed in the boxes. Remember to include a breakpoint if two *Hold until* instructions are used in the same phase. In this example method the Base has been set to Volume if not specified otherwise.

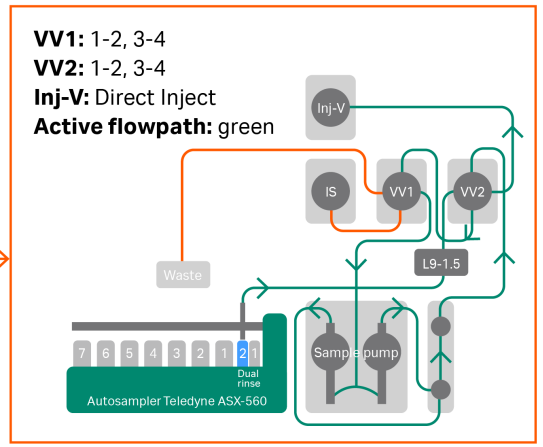
```
0.00  Phase: Teledyne control - Pump On
    0.00  Base: SameAsMain
    0.00  Hold until: Digital in 4, Equals, 1, Infinite (base)
    0.00  Pulse digital out 4: 0.5 (sec)
    Comment: Pulse Digital out 4: Turn On ASX560 Pump.
    0.00  End_Block
0.00  Phase: Wash Probe - Rinse station 1 NaOH
    0.00  Base: SameAsMain
    0.00  Pump wash: Off, Off, Off, S1
    0.00  End_Block
0.00  Phase: Teledyne control - Pump Off
    0.00  Base: SameAsMain
    0.00  Pulse digital out 3: 0.5 (sec)
    Comment: Pulse Digital out 3: Turn Off ASX560 Pump.
    0.00  End_Block
0.00  Phase: Teledyne control - Pump On
    0.00  Base: SameAsMain
    0.00  Hold until: Digital in 4, Equals, 0, Infinite (Base)
    0.00  Pulse digital out 4: 0.5 (sec)
    Comment: Pulse Digital out 4: Turn On ASX560 Pump.
    0.00  End_Block
0.00  Phase: Wash Probe - Rinse station 2 Buffer
    0.00  Base: SameAsMain
    0.00  Pump wash: Off, Off, Off, S1
    0.00  End_Block
0.00  Phase: Teledyne control - Pump Off
    0.00  Base: SameAsMain
    0.00  Pulse digital out 3: 0.5 (sec)
    Comment: Pulse Digital out 3: Turn Off ASX560 Pump.
    0.00  End_Block
0.00  Phase: System Wash on Bypass to remove NaOH
    0.00  Base: SameAsMain
    0.00  Column position: By-pass, Down flow
    0.00  System wash: 75 (ml), Outlet valve
    0.00  End_Block
```

Flush out configuration – Sample application

Add three **User Defined** phases to the method and rename them.
In this example method the Base has been set to Volume if not specified otherwise.

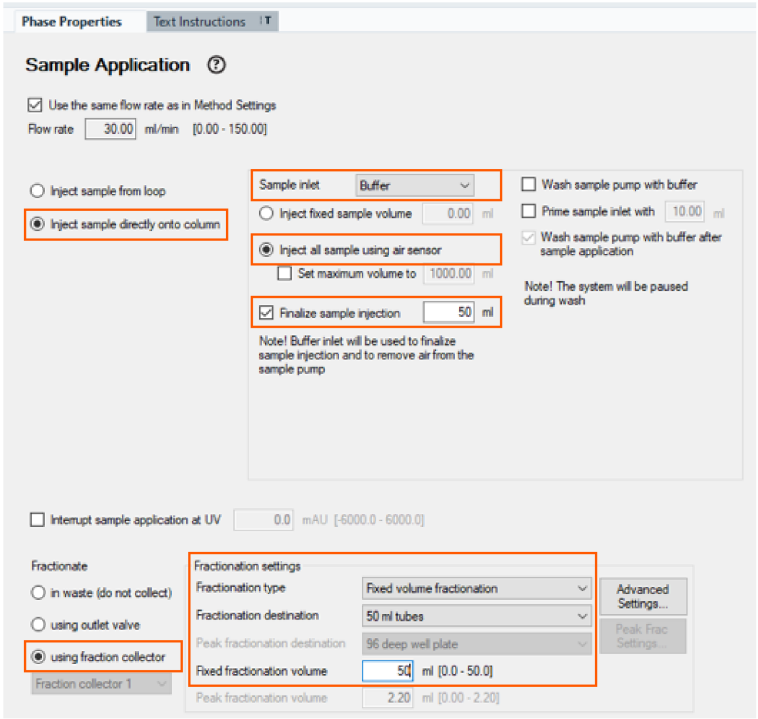


Sample injection flowpath

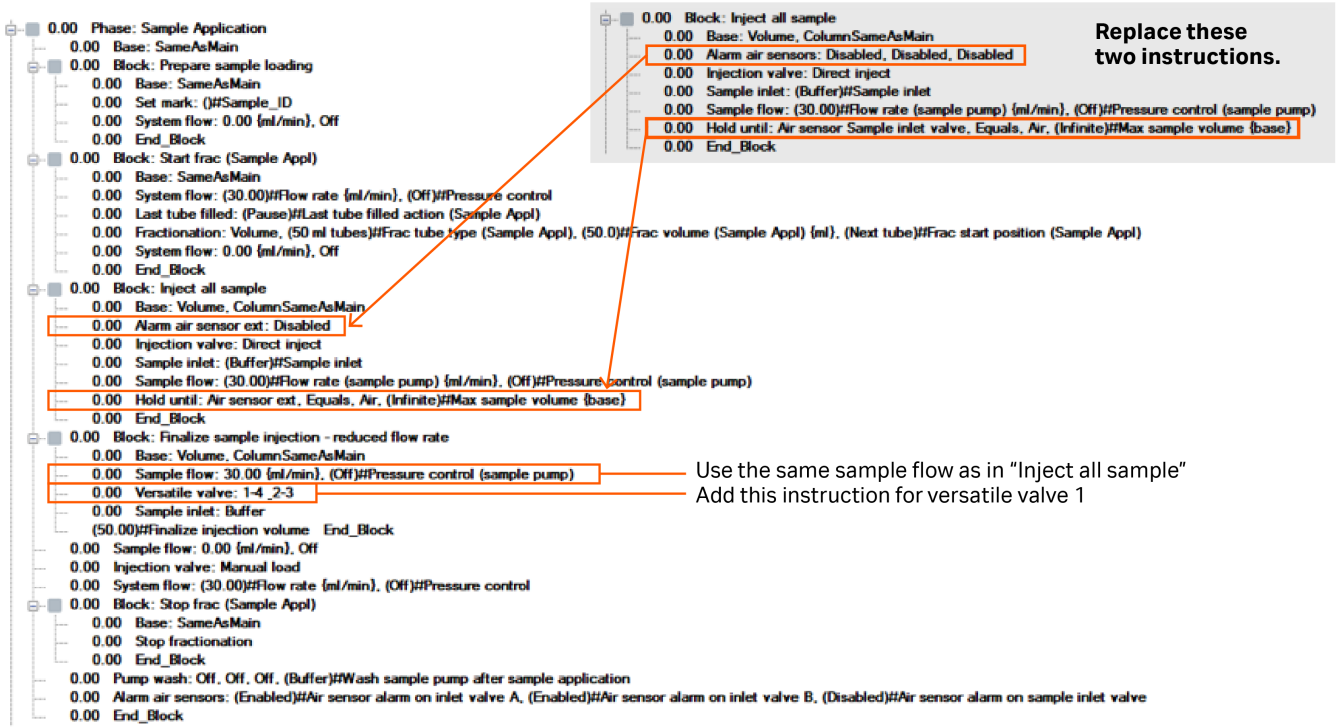


Phase: **User Defined**. In Text Instructions, open the tree and add the instructions to each User Defined phase, as indicate by the boxes. The instructions are found in the Instruction Box. Remember to include a breakpoint for the second *Hold until* instruction.

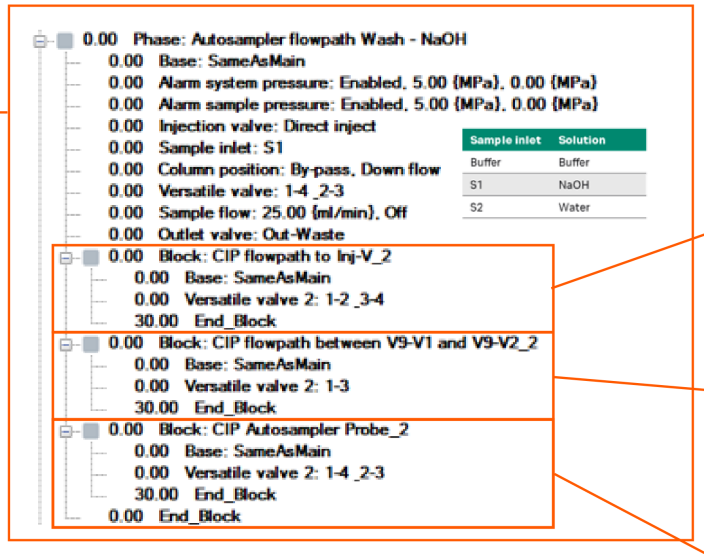
Phase: **Sample Application**. In **Phase Properties**, start by select the instructions as indicated by the boxes.



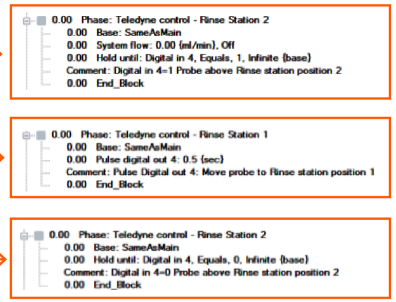
Phase: **Sample Application**. In **Text Instructions**, open the tree and replace the two instructions marked in the boxes in “Inject all sample”. In “Finalize sample injection – reduce flow rate” add the instruction as indicated in the box.



Flush out configuration – CIP of the probe



Sample inlet	Solution
Buffer	Buffer
S1	NaOH
S2	Water



The UNICORN™ method for the CIP of the *Flush out configuration* consist of Teledyne control phases and Autosampler flowpath Wash phases. Each Autosampler flowpath Wash phase uses one rinse solution and cycles through the three different flowpaths as described by the wash sequence to the right.

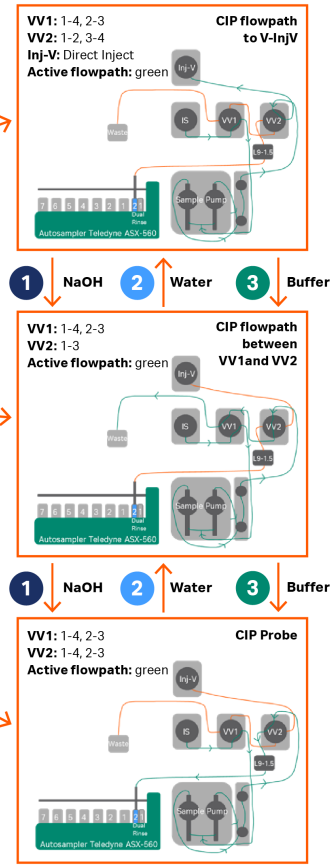
For each wash sequence select the corresponding sample inlet and use an appropriate volume for each step (defined by the End block volume).

These volumes are approximate and might be subject to change if buffering solutions are used.

Modifying the number of wash solution will affect the order of wash sequence execution.

Block	End block volume (mL NaOH)	End block volume (mL Water)	End block volume (mL Buffer)
CIP flowpath to Inj-V	30	75	75
CIP flowpath between V9-V1 and V9-V2	30	50	50
CIP Autosampler Probe	30	50	75

Wash sequence



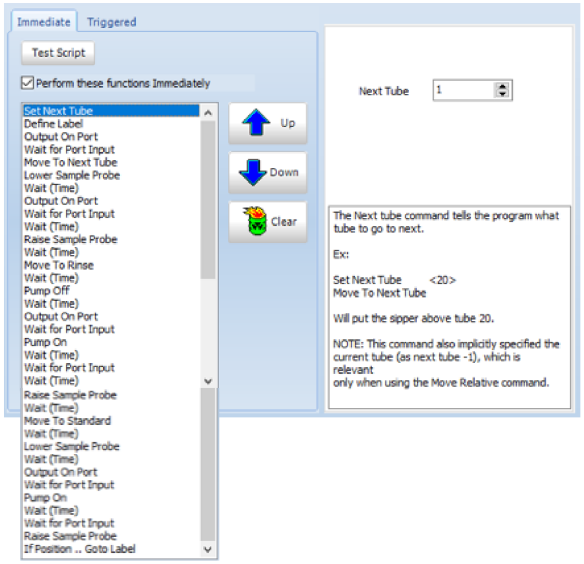
AScript

When creating scripts in AScript the available commands are displayed in a list on the left-hand side of the window. Either drag or double click on a command to place it in the script. The command can be moved up or down but not deleted from the script. The clear button deletes an entire script. Make sure to save the script.

To delete a specific instruction in the AScript method, mark the instruction and use the delete button on the keyboard.

Standard configuration – Sample application and CIP of the probe

In **AScript**, select **Immediate** and add the commands as in the order below. Go through each command and update the instructions according to the second column in the table.

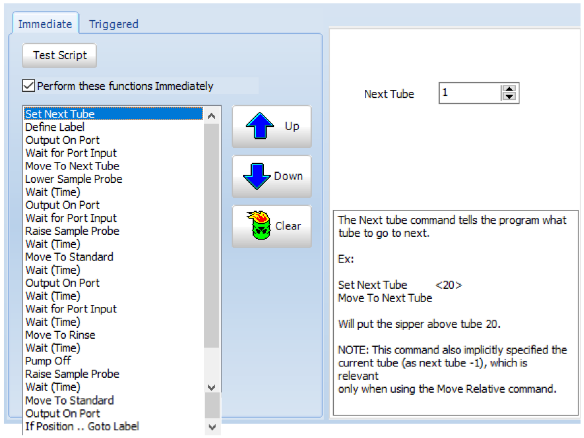


The first two columns in the table gives an overview of each AScript instruction. The last two columns describes which UNICORN™ instruction that synchronize with the AScript command and explains the instruction.

AScript - Immediate		UNICORN™	Description
Set Next Tube	1		
Define Label	Start		AScript returns to this point to loop a cycle
Output On Port	1 – Disabled (Duration 0 ms)	Digital in 1 =1	Ready for Next Sample
Wait for Port Input	1 – Open	Pulse Digital out 1	Move Probe to Next Sample
Move To Next Tube	-		
Lower Sample Probe	140 mm		
Wait (Time)	5		
Output On Port	1 – Enabled (Duration 0 ms)	Digital in 1 = 0	Probe in Next Sample Tube
Wait for Port Input	2 – Open	Pulse Digital out 2	Sample application finished
Wait (Time)	5		
Raise Sample Probe	-		
Wait (Time)	5		
Move To Rinse	140 mm		
Wait (Time)	5		
Pump Off	-		
Wait (Time)	5		
Output On Port	4 – Disabled (Duration 0 ms)	Digital in 4 = 1	Probe in Rinse station 1
Wait for Port Input	4 – Open	Pulse Digital out 4	Turn on the pump. Ready for S1 pump wash using rinse station 1 (NaOH)
Pump On	-		
Wait (Time)	5		
Wait for Port Input	3 - Open	Pulse Digital out 3	Turn off the pump after the S1 pump wash using rinse station 1 (NaOH).
Wait (Time)	5		
Raise Sample Probe	-		Also turns off the pump
Wait (Time)	5		
Move To Standard	1		Rinse station position 2.
Wait (Time)	5		
Lower Sample Probe	140		
Wait (Time)	5		
Output On Port	4 – Enabled	Digital in 4 = 0	Probe in Rinse station 2
Wait for Port Input	4 – Open	Pulse Digital out 4	Turn on the pump. Ready for S1 pump wash using rinse station 2 (Buffer)
Pump On	-		
Wait (Time)	5		
Wait for Port Input	3 – Open	Pulse Digital out 3	Turn off the pump after the S1 pump wash using rinse station 2 (Buffer).
Raise Sample Probe	-		Also turns off the pump
If Position.. Goto Label	< Last Tube Goto Label Start Use Run-Time Comparison Position		This command loops the script back to the Start label

Flush out configuration – Sample application and CIP of the probe

In **AScript**, select **Immediate** and add the commands as in the order below. Go through each command and update the instructions according to the second column in the table.



The first two columns in the table gives an overview of each AScript instruction. The last two columns describes which UNICORN™ instruction that synchronize with the AScript command and explains the instruction.

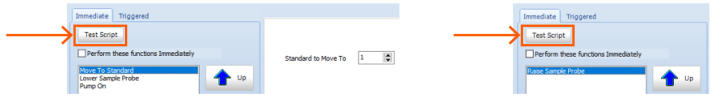
AScript - Immediate		UNICORN™	Description
Set Next Tube	1		
Define Label	Start		AScript returns to this point to loop a cycle
Output On Port	1 – Disabled (Duration 0 ms)	Digital in 1 =1	Ready for Next Sample
Wait for Port Input	1 – Open	Pulse Dig out 1	Move Probe to Next Sample
Move To Next Tube	-		
Lower Sample Probe	140 mm		
Wait (Time)	5		
Output On Port	1 – Enabled (Duration 0 ms)	Digital in 1 = 0	Probe in Next Sample Tube
Wait for Port Input	2 – Open	Pulse Digital out 2	Sample application finished
Raise Sample Probe	-		
Wait (Time)	5		
Move To Standard	1		
Wait (Time)	5		
Output On Port	4 – Disabled (Duration 0 ms)	Digital in 4 =1	Probe in Rinse station position 2
Wait (Time)	5		
Wait for Port Input	4 – Open	Pulse Digital out 4	Move Probe to Rinse station position 1
Wait (Time)	5		
Move to Rinse	-		
Wait (Time)	10		The time for the outside wash of the probe
Pump Off	-		
Raise Sample Probe	-		
Wait (Time)	5		
Move To Standard	1		
Output On Port	4 – Enabled (Duration 0 ms)	Digital in 4 =0	Probe in Rinse station position 2
If Position.. Goto Label	< Last Tube Goto Label Start Use Run-Time Comparison Position		This command loops the script back to the Start label

How to start a run

How to start the methods

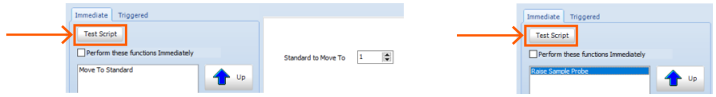
- Before starting the methods, make sure that all four **Digital out** and **Digital in** signals in UNICORN™ have the value 0.
- Prepare and fill the ÄKTA™ system with appropriate buffer.
- Prepare and fill the sample probe flowpath with appropriate buffer:

  – ***Standard configuration:*** In **AScript**, move the probe to rinse station position 2 (Buffer) and perform a sample pump wash with S1 in UNICORN™.



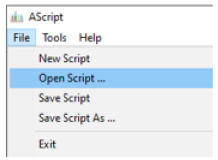
Start a Sample pump wash for S1 in UNICORN™

  – ***Flush out configuration:*** In **AScript** move the probe to rinse station position 2, ***Move to Standard 1.*** In UNICORN™ perform a **manual run** and fill the flowpaths and sample probe, as described in the **CIP of the probe - Wash sequence**, using the **Buffer inlet**.

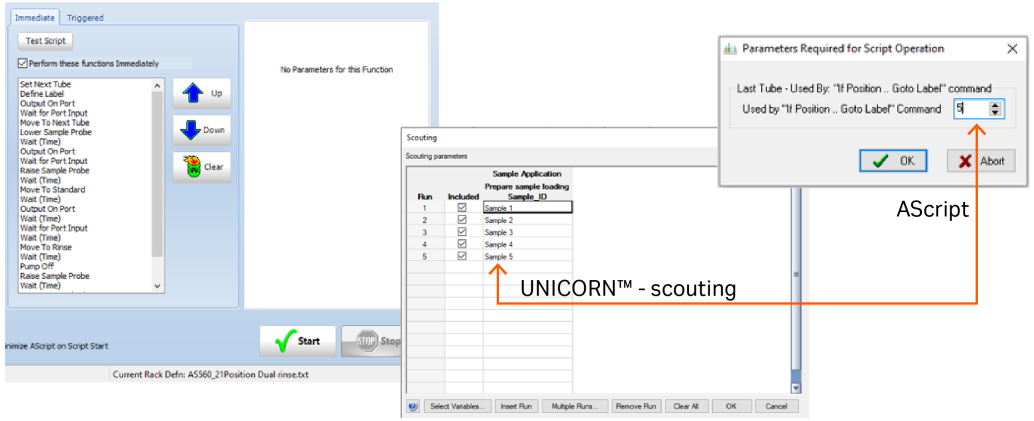


Manual run in UNICORN™, follow the CIP of the probe Wash sequence, use the Buffer inlet.

- Start the AScript method:



In **AScript** select **File** and **Open Script**. Select the script compatible with the active setup (*Standard- or Flush out configuration*). The method will be displayed in the **Immediate** tab. Click **Start** and select the **number of samples** included in the run. The number of samples should correspond to the number of samples included in the UNICORN™ method (scouting scheme).



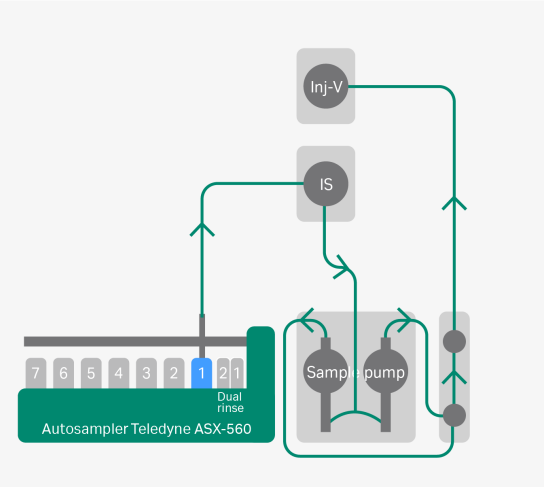
- Start the UNICORN™ method, make sure the number of samples in the scouting scheme correspond to the number of samples specified when starting the AScript method.

6

Flowpath during method progress

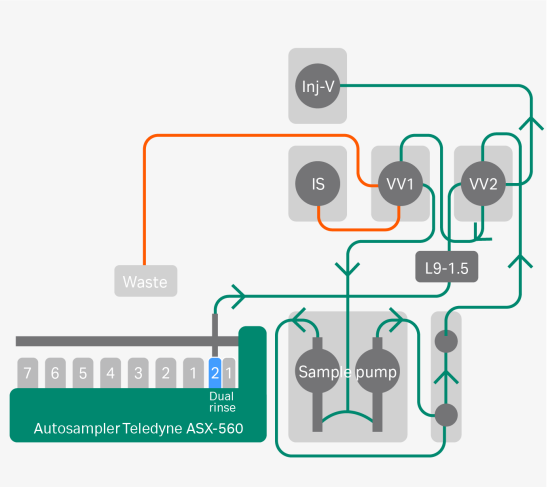
Sample flow path in the standard configuration

Sample injection
IS: Probe connected to S1
Active flowpath: green

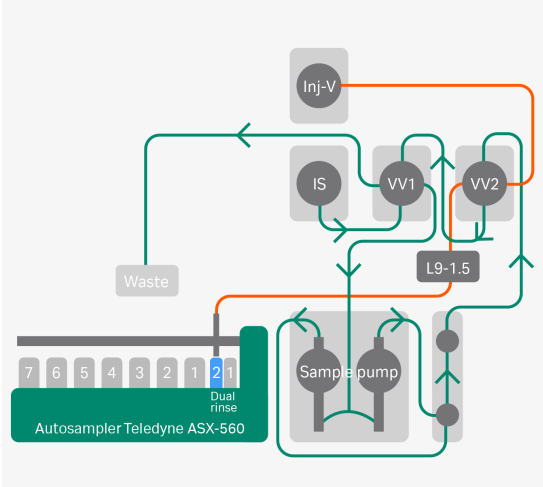


Flow path in the Flush out configuration

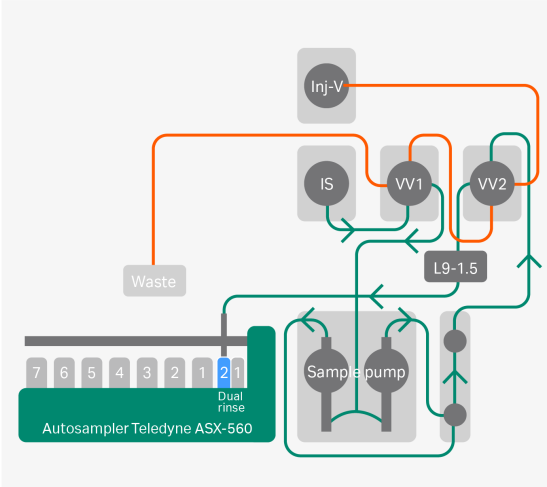
Sample injection
VV1: 1-2, 3-4
VV2: 1-2, 3-4
Inj-V: Direct Inject
Active flowpath: green



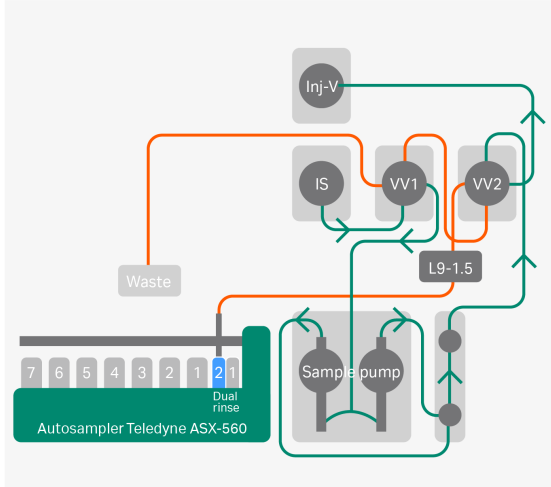
CIP flowpath between VV1 and VV2
VV1: 1-4, 2-3
VV2: 1-3
Active flowpath: green



CIP Probe
VV1: 1-4, 2-3
VV2: 1-4, 2-3
Active flowpath: green



CIP flowpath to V-InjV
VV1: 1-4, 2-3
VV2: 1-2, 3-4
Inj-V: Direct Inject
Active flowpath: green



To optimize the flowpath minimize tubing length as possible.

7

Appendix

Node IDs

Node IDs are component specific numbers used to identify the modules used in the ÄKTA™ configurations. They are printed on the back of each module.

Module	Label		Node ID
Versatile valve	V9-V or V9H-V	VV1	20
Versatile valve 2	V9-V or V9H-V	VV2	21
External air sensor	L9-1.5		0
I/O box	E9		0

Thank you

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