
User Guide

Biochrom Alias

Autosampler

Please read this user guide carefully before using the Autosampler



CE

Description of Version Changes:

Date	CR No	Version	List of Changes
30 APR 2022	DCN0031	01	Initial Release

Warranty and Liability:

Biochrom Ltd guarantee that the product supplied has been thoroughly tested to ensure that it meets its published specification. This warranty may only be valid if the product has been used within its specification and in all respects has been operated and maintained in a normal, proper manner in accordance with the Instruction Manual. The Alias Autosampler System hardware is guaranteed for a period of 12 months from date of installation or 15 months from date of dispatch, whichever is sooner. The general warranty conditions may not be valid if the defect is due to accident, unauthorized modification, unauthorized repair attempt, incorrect operation or transport damage. Biochrom Ltd can accept no liability for loss or damage, however caused, arising from the faulty or incorrect use of this product.

Abbreviation of Terms:

Terms	Abbreviation
RSD	Relative Standard Deviation
HPLC	High-Performance Liquid Chromatography
PASA	Pressure-Assisted Sample Aspiration
BAM	Biochrom Alias Manager
IPA	Isopropyl Alcohol/Isopropanol
PEEK	Polyether ether ketone

Part Number:

Part Number	Description
40014706	AUTOSAMPLER - BIO30 PLUS LEAD
41700079	ALIAS AUTOSAMPLER BIO + COOL

Manufacturer Details:

Any technical assistance regarding the Autosampler, its accessories and consumables should be addressed to:

Biochrom Limited, Unit 7, Enterprise Zone

3970 Cambridge Research Park

Beach Drive, Waterbeach

Cambridge, United Kingdom, CB25 9PE

E-mail : support@biochrom.co.uk

Tel: +44 (0)1223 423723



Authorised Representative

Medical Device Safety Service GmbH (MDSS)

Schiffgraben 41, Hannover 30175

Germany

Serious Incidents

Serious incident that has occurred in relation to the device shall be reported to the manufacturer/authorized representative and the competent authority of the member state in which the user and/or the patient is established.



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About this guide

This guide is written for laboratory technicians who use the Alias autosampler for execution of analytical runs.

This guide offers the following information:

- Chapter 2 describes the Alias autosampler and injection principles
- Chapter 3 describes installation and upgrading procedures for the Alias autosampler and Biochrom Alias Manager software
- Chapter 4 describes maintenance procedures for the Alias autosampler
- Chapter 5 describes trouble shooting
- Appendix A lists specifications of the Alias autosampler
- Appendix B provides information on Control I/O
- Appendix C lists error messages
- Appendix D lists accessories and spare parts available for the Alias autosampler.

An index has been provided for easy reference.

Pictorials used in this manual

The following pictorials are used in this guide:



The danger sign warns about a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in injury or loss of life.

Do not proceed beyond a danger sign until the indicated conditions are fully understood and met.



The warning sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in severe injury or damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.



The caution sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in damage or destruction of parts or all of the equipment. Do not proceed beyond a cautions sign until the indicated conditions are fully understood and met.



The attention sign signals relevant information. Read this information, as it might be helpful.



The note sign signals additional information. It provides advice or a suggestion that may support you in using the equipment.

Safety Practices

The following safety practices will ensure safe operation of the autosampler and should only be executed by authorized personnel:



Removal of some panels exposes potentially dangerous voltages. Disconnect the instrument from all power sources before removing protective panels.

Replace blown fuses with size and rating indicated on the fuse panel or holder and as listed in the list of accessories and spares (appendix D) in this manual.

Replace or repair faulty or frayed insulation of power cords.

Check actual line voltage to confirm it is the value for which this instrument is wired. Make sure power cords are plugged into the correct voltage sources.



Perform periodic leak checks on supply lines.

Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.



Using this instrument in other ways than described in this guide or defined by Good Laboratory Practice may result in unsafe operation.

Introduction to Alias Autosampler

Intended Use: The Alias Autosampler is fully automated laboratory instrument, intended to be used along with the Bio30+ Amino Acid Analyser as an aid to the diagnosis of phenylketonuria by trained professionals. The Alias Autosampler is designed for the consistent injection of samples in the fully automatic Bio30+ Amino Acid Analyser system, to provide quantitative analysis of phenylalanine and tyrosine present in physiological samples. Physiological samples of human origin include, but are not limited to, blood, urine, cerebrospinal fluid, and amniotic fluid. The Alias Autosampler is not intended to be used as a standalone diagnostic test for any patient condition or disease and is for in vitro use only. Alias autosampler can be manually controlled with Alias Manager software.

The Alias autosampler is a high throughput autosampler of robust design, developed to meet the challenge of the modern analytical laboratory. It is a very complete autosampler that needs little bench space; the Alias autosampler is designed for indoor use. The Alias autosampler features among other things:

- PASA™ **injection concept** (see "Injection Principles" on page 14).
- High-resolution syringe control: this ensures very high precision for injection and reagent addition.
- PC control ensures easy-to-understand operation.
- Special attention has been paid to ensure a service-friendly design.
- To enhance safety, speed of operation of the Alias autosampler will decrease when the door is opened.
- Sample cooling ensures consistent results.

Read this chapter to help identify parts of the Alias autosampler, and to learn more about injection principles.



Close the Autosampler door during its operation

Instrument description

The Alias autosampler is standard fitted with:

- 15 μL injection needle
- 500 μL syringe
- 1000 μL buffer tubing
- 200 μL sample loop.

Opening the door: To open the door, execute the following steps

- 1 Get hold of the door handle.

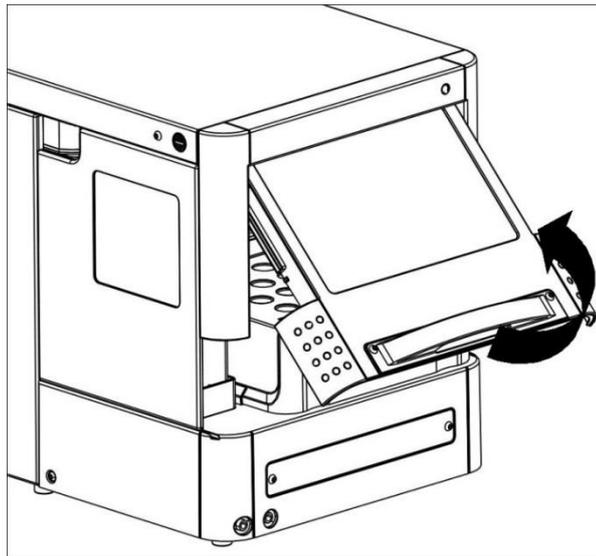


Figure 1: Open the door

- 2 Gently pull it towards you and push it upward until it is in horizontal position.

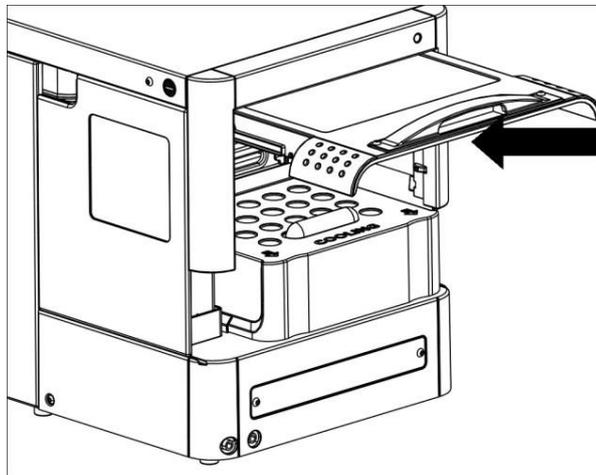
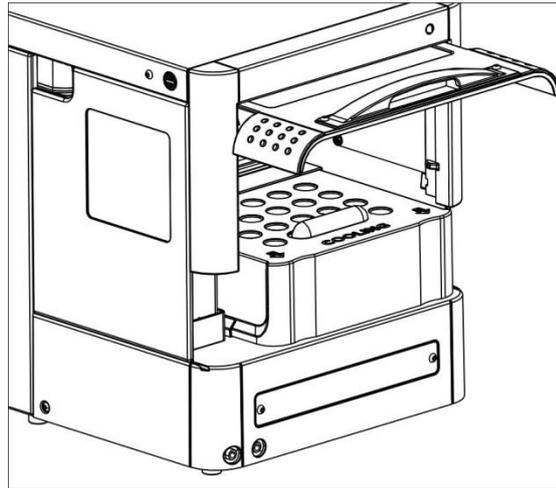


Figure 2: Push the door upward

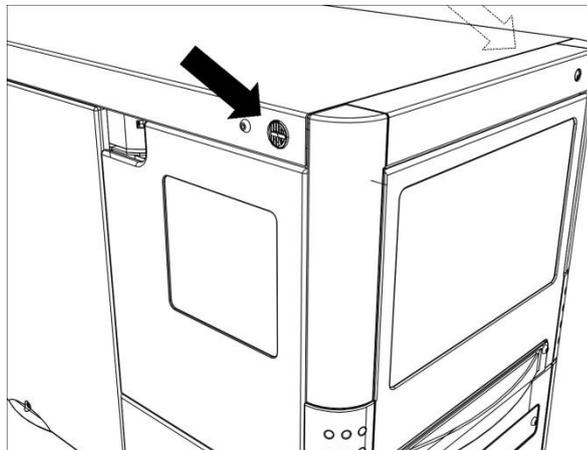
- 3 Slide the door into the autosampler.

Figure 3: Slide the door into the autosampler



Removing the cover: For easier access, you can remove the cover of the Alias

Figure 4: Location of Black push buttons

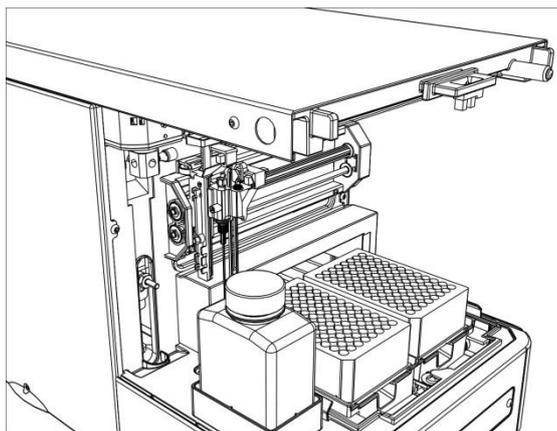


To remove the cover of the Alias:

- 1 Press the two black buttons on either side (top) of the autosampler simultaneously.

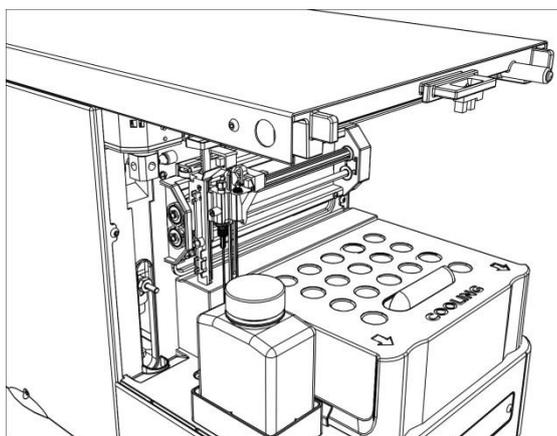
2 Gently pull the cover towards you.

Figure 5: Alias without cover



If the cooling option is installed: slide out the cooling cover by pulling it gently towards you. You can now place well plates.

Figure 6: Alias with cooling cover



Alias autosampler - front

The Alias sampling compartment houses the following parts:

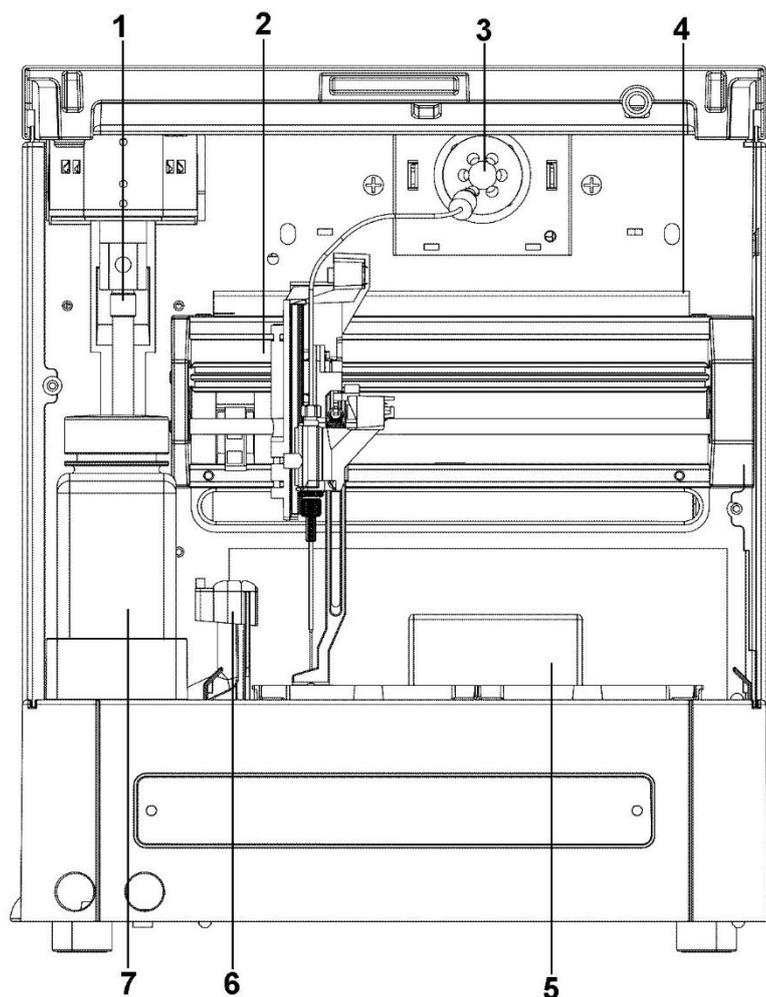
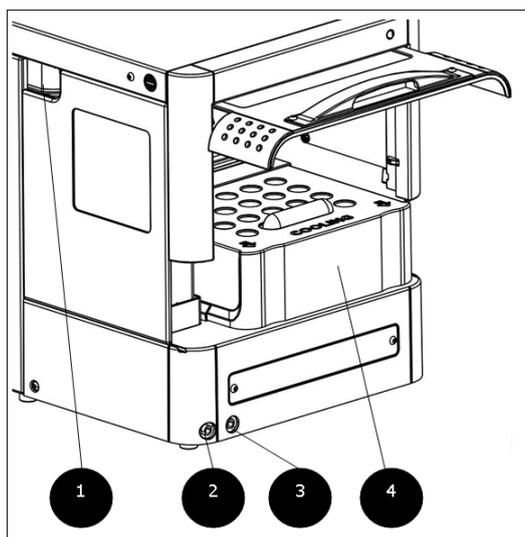


Figure 7: Alias sampling compartment

- 1 Syringe
- 2 Needle arm
- 3 Injection valve
- 4 Valve leak bin
- 5 Sample compartment
- 6 Needle wash position
- 7 Wash liquid bottle

Figure 8: Alias with cooling

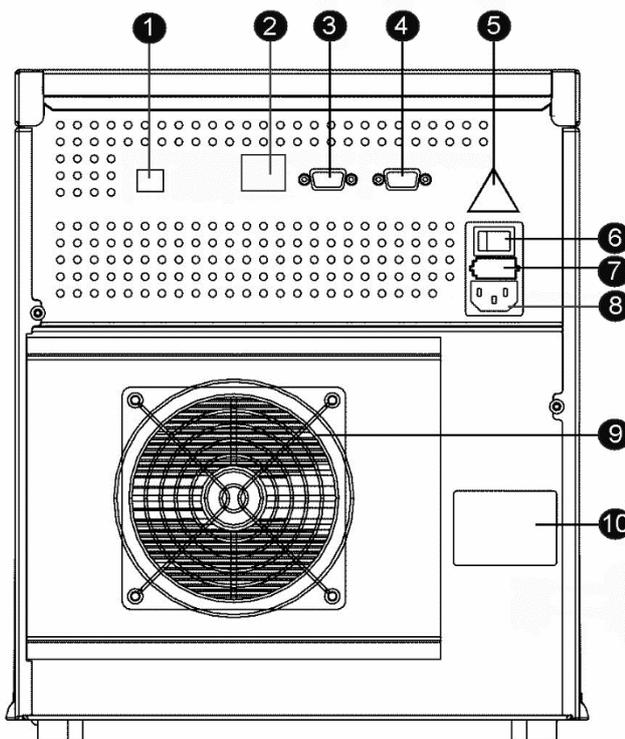


- 1 Tubing guide
- 2 Wash/waste
- 3 Condensed water/leakage
- 4 Cooling cover

Alias autosampler - back

The back of the autosampler has the following items:

Figure 9: Alias (back)



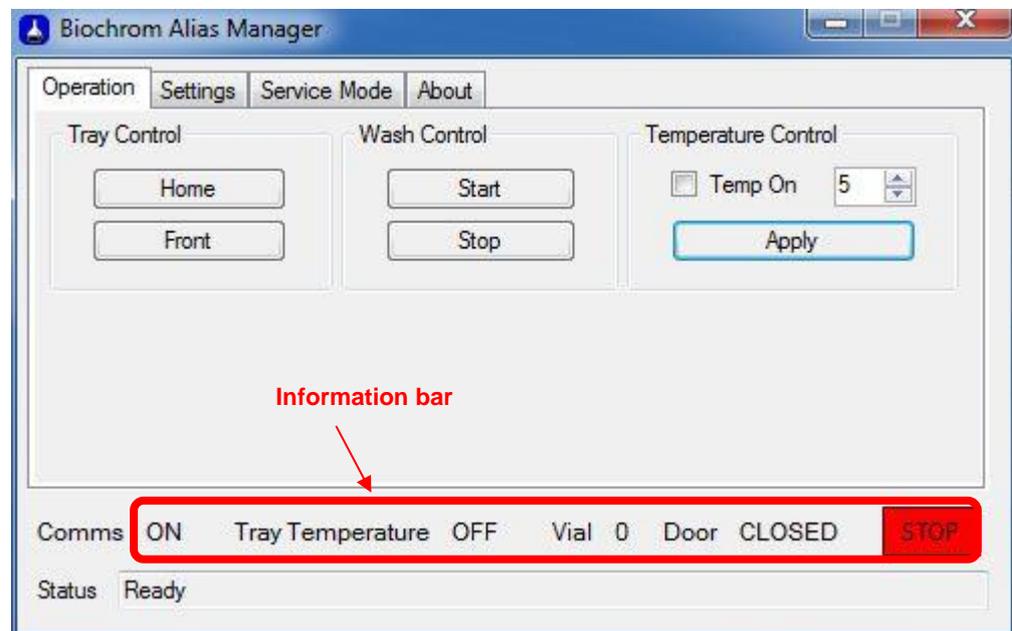
- 1 USB connector (optional)

- 2 Ethernet connector (optional)
- 3 9-pin male connector (inputs/output)
- 4 9-pin female connector (serial interface)
- 5 **warning label** (see "Control I/O connections")
- 6 on/off switch
- 7 fusebox
- 8 power connector
- 9 cooling fan (if cooling option is installed; do not obstruct!)
- 10 type label

{“Alias Manager Software Description”}

Alias Manager Software Description

Operation tab



Tray control:

- Click on **Front** to move the sample tray forward. This allows the access to the samples and enables the removal of the tray from the compartment.
- Click on **Home** in order to move the tray back in its default position

Wash control:

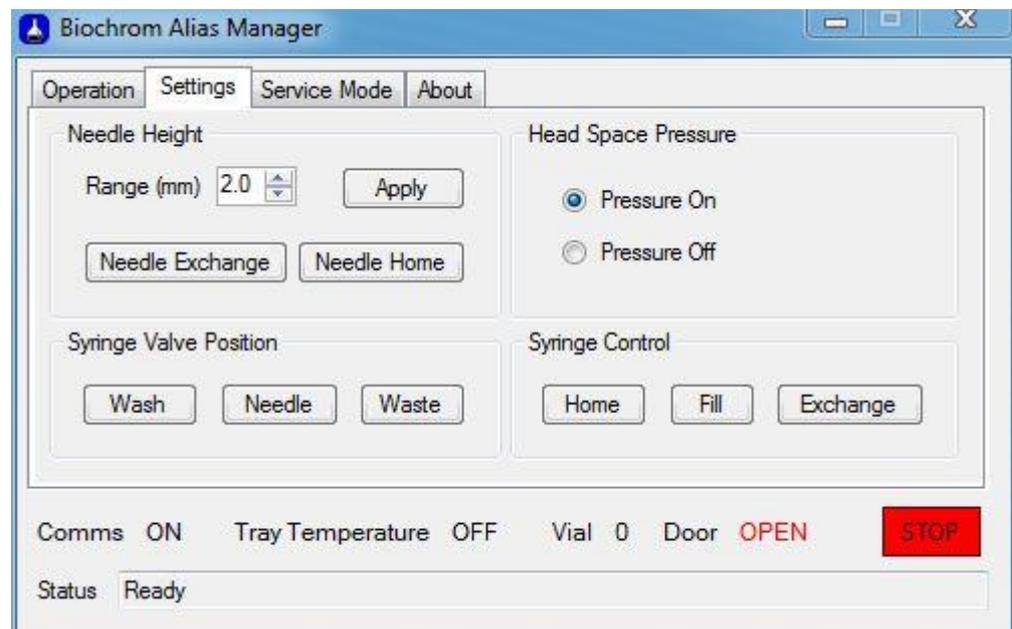
- Click on **Start** to perform a wash sequence of the needle and the syringe

- Click on **Stop** if you wish to stop the wash sequence before the end of a full cycle.

Temperature control:

- Set the temperature required in the temperature box
- Tick the **Temp ON** box and click **apply** to enable the cooling system to start.
- Once the cooling system has started, the actual temperature tray will be displayed in the information bar. If the temperature is in red, it means the actual temperature does not match the required temperature. Once the required temperature has been reached, the tray temperature will be displayed in black.

Settings tab



Needle height:

- Range: Select the distance in mm between the bottom of the vial and the needle. Click apply to record the change.
- Needle Exchange: click on this button to position the needle in the exchange position.
- Needle Home: Once the needle has been changed, click on this button to reinitialise the needle position.

Head space pressure:

- Pressure On: select this option when vials with septa are used.
- Pressure Off select this option when no caps are used on the vials.

Syringe valve position

- Wash: move the valve in the wash position
- Needle: move the valve to divert the flow to the needle
- Waste: move the valve to divert the flow to the waste

Syringe control:

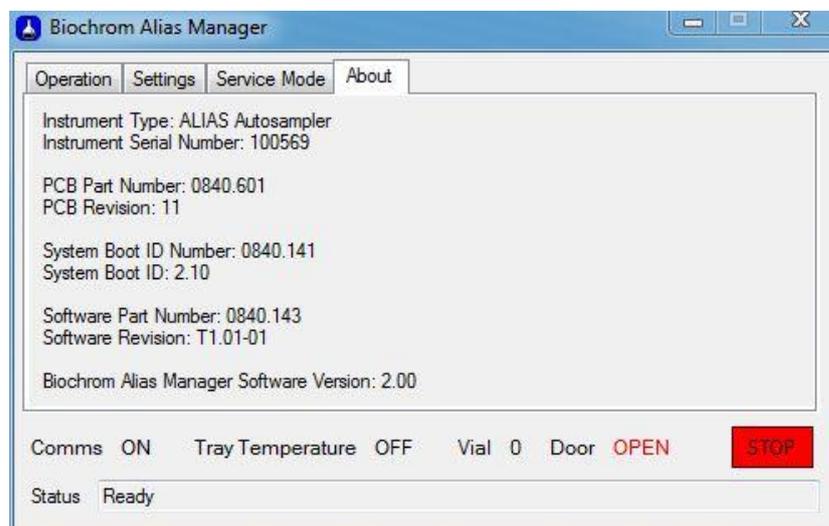
- Home: position the syringe in the home position
- Fill: fill the syringe with the washing liquid
- Exchange: move the syringe in the exchange position

Service mode tab



This tab is only accessible for service engineers with a suitable password.

About tab



This tab shows the instrument type, serial number and software version details.

Minimized window



This window is triggered when the minimize button is clicked on the main Alias Manager window. It contains all the basic information along with main operations buttons. To recover the full window of the Alias Manager, click on **Maximize**.

Options

The following **factory-installed** options are available for the Alias autosampler:

- Cooling: if installed, a cooling fan is visible at the back of the autosampler, and a cooling cover is installed inside the sampling compartment.
- Bio-compatible sample flow path and valve Inert sample needle (Silco steel) and bio-compatible valve (PEEK)

Injection principles

Three injection modes can be used:

- **Full loop injections:** for maximum precision
- **Partial loopfill injections** (on page 19): for maximum flexibility
- **µL Pickup injections** (on page 22): for zero sample loss.

These three injection modes accommodate use of a wide variety of applications.

For all injection modes loop injection with Pressure-Assisted Sample Aspiration (PASA™) is selectable. It is a proven concept that combines high precision with simplicity and reliability:

- no moving around with the sample needle
- reduced risk for bubbles in the sample line
- no needle port that wears and contaminates.

There is only intelligent valve switching and highly accurate syringe control.

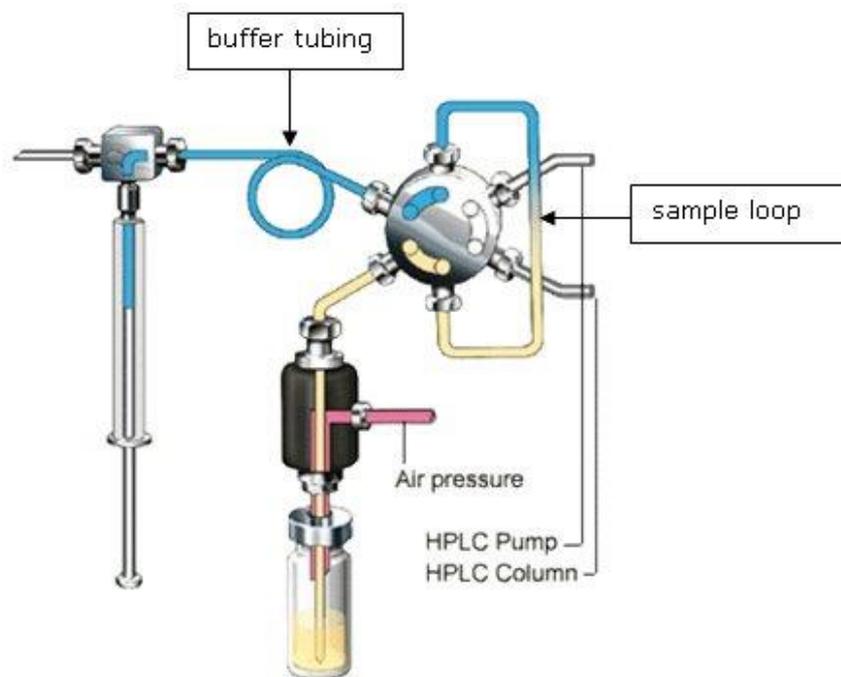


Figure 10: PASA injection concept

The syringe is used to aspirate the sample from a vial into the sample loop. Buffer tubing between the syringe and the injection valve prevents contamination of the syringe. Wash solvent is used:

- to remove the sample from the buffer tubing and sample needle
- to rinse the buffer tubing and sample needle.



Wash the injection needle after every analysis

Syringe and buffer tubing Alias

Three sizes of syringes are available for the Alias: 250, 500 and 2500µL. The 2500µL syringe is installed in the Prep version of the Alias.

The 500µL syringe is the standard syringe, combined with the 1000µL buffer and the standard 200µL sample loop. The following injection volume ranges are available for the various injection modes:

-
- Full loop : 200 µL
 - Partial loopfill : 0 - 100 µL
 - µL pick-up : 0 - 77µL

The maximum injection volumes are calculated with the following formulas:

- Full loop : injection volume = loop volume
- Partial loopfill : max. inj. volume = $\frac{1}{2}$ of loop volume
- µL Pick up : max. inj. volume = (loop volume - 3 x needle volume)/2

Full loop gives maximum possible reproducibility < 0.3%, but not maximum accuracy, since the loop volume is specified with an accuracy of $\pm 10\%$. Minimum sample loss = 230 µL (2 x loop overfill + flush volume for 15 µL needle).

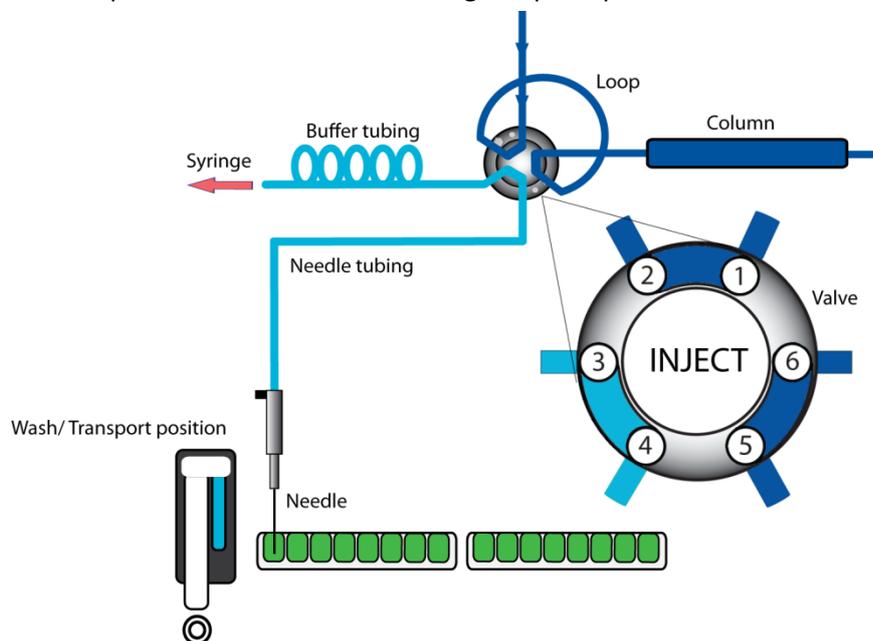
Partial loopfill gives maximum accuracy plus reproducibility better than 0.5% RSD for injection volumes > 5 µL. Minimum sample loss (Flush volume) = 30 µL. 30 µL is the recommended minimum flush volume, smaller flush volumes can be programmed, but will result in decreasing performance.

µL Pick-up offers no sample loss, maximum accuracy (same as partial loopfill), but slightly lower reproducibility: RSD better than 1% for injection volumes > 5 µL.

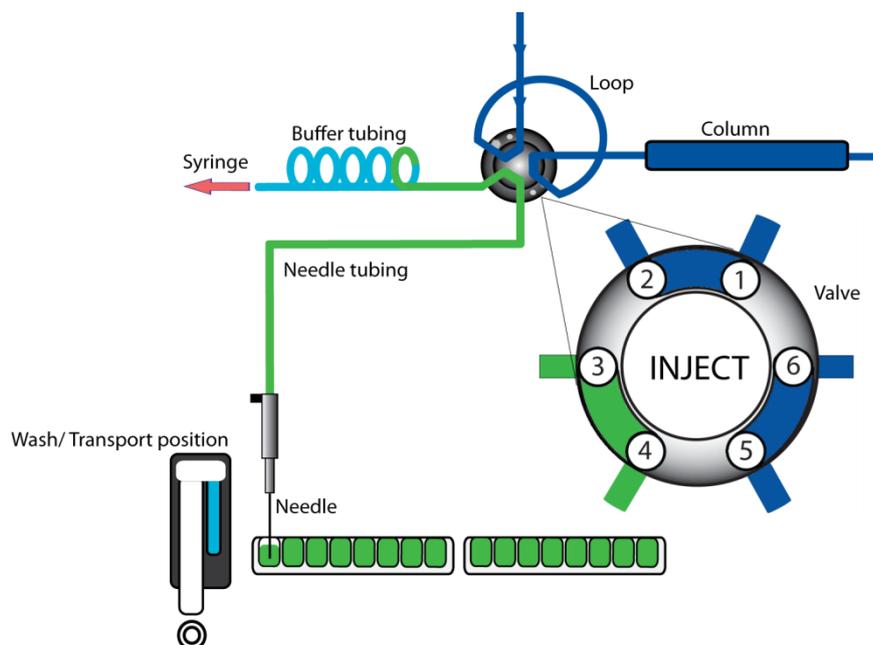
Full loop injections

The sample loop is completely filled (quantitatively) with sample. This type of injection results in extremely good reproducibility.

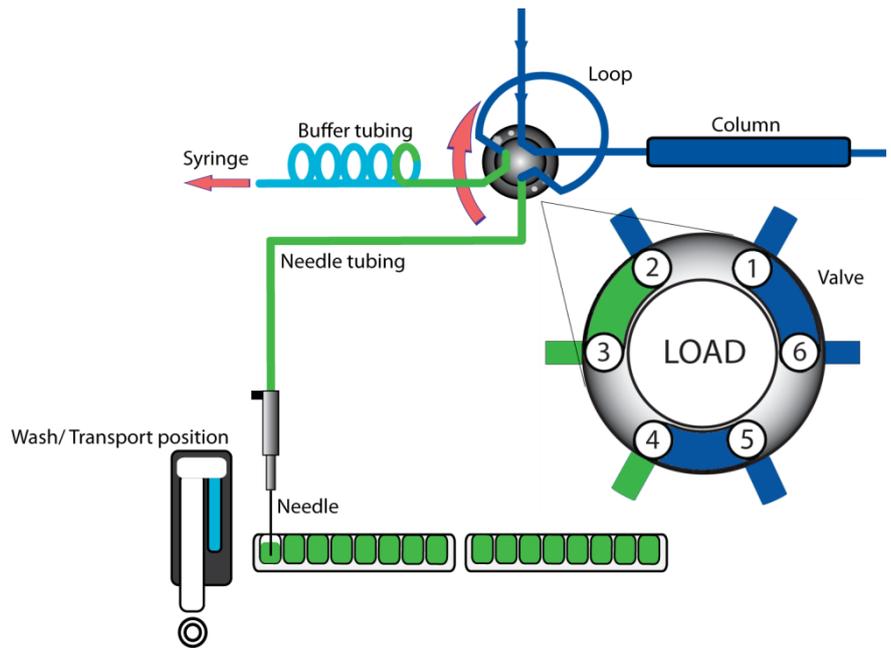
- 1 The initial situation: the injection valve is in INJECT position. The sample needle with air needle has entered the well or vial. Headspace pressure, applied through the air needle, ensures that no air or vapor bubbles are formed during sample aspiration.



- 2 The syringe dispenser aspirates the "flush volume" from the sample well/vial to fill the sample line with sample and remove wash solvent.



- 3 The injection valve is switched to LOAD position, placing a distinct sample plug at the inlet of the sample loop.

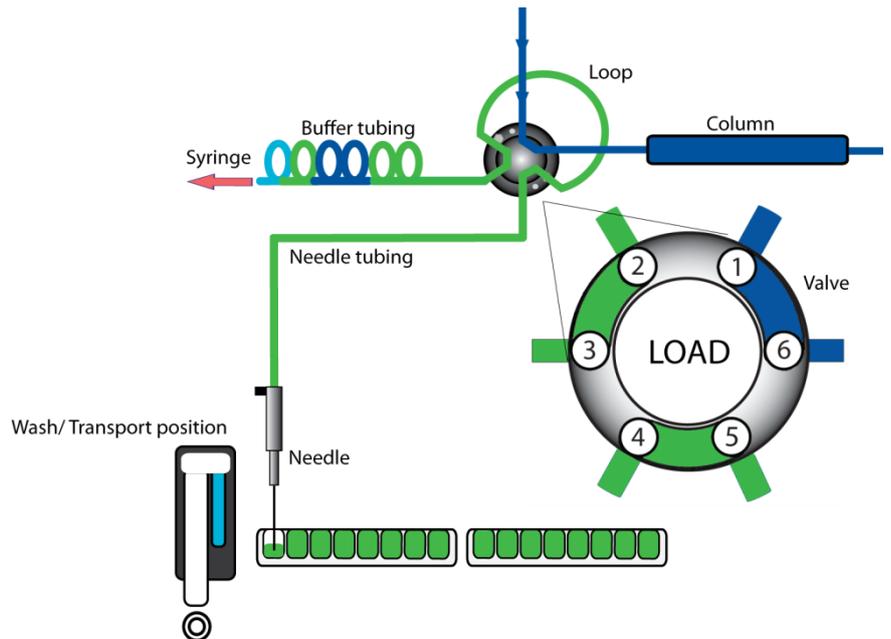


- 4 The sample loop is quantitatively filled by transporting a number of times the loop volume through the loop, depending on the volume of the loop.

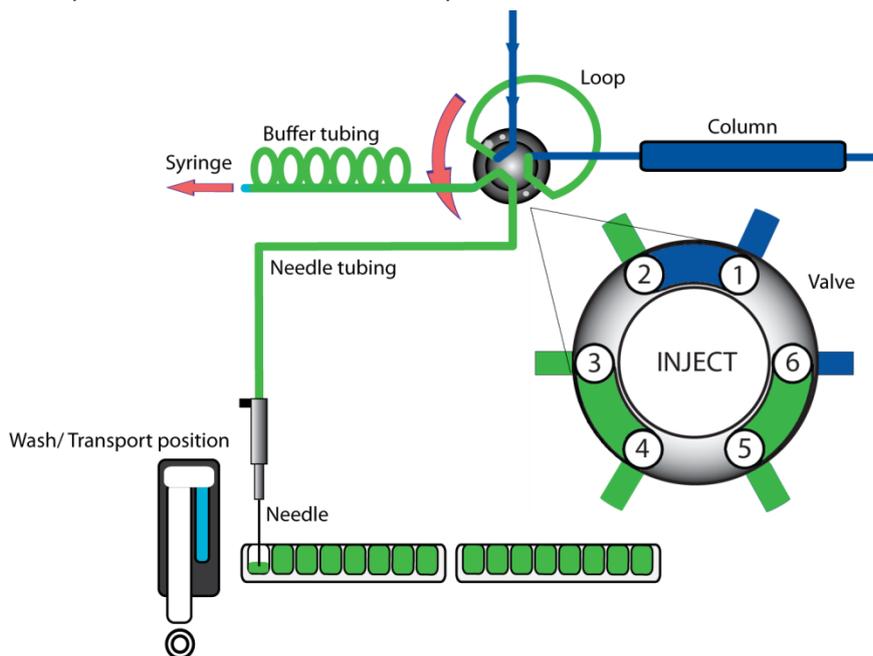
3 x loop volume for loop \approx 100 μ L

2 x loop volume for loops 100 μ L - 500 μ L

1.5 x loop volume for loop \approx 500 μ L



- 5 The injection valve switches to the INJECT position. The sample loop is not part of the (U)HPLC mobile phase flow path: sample is transported to the column. The analysis starts.

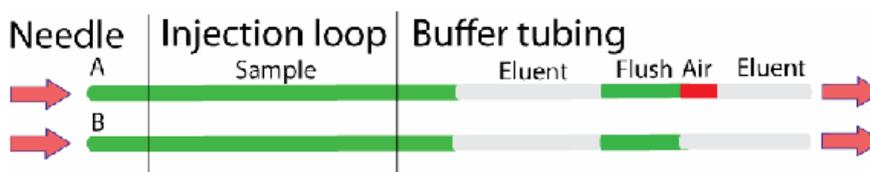


A wash routine is performed after each injection.

Air segment with Full loop injections

An air segment of 5 μL can be used to reduce the amount of flush volume. This air segment is at the front of the flush volume and will not be injected.

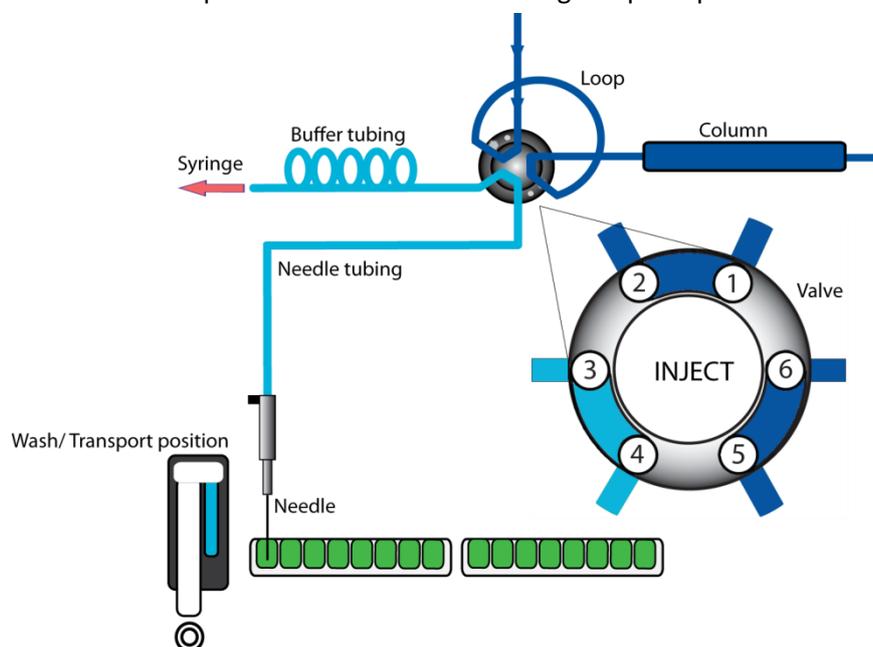
With a standard needle, the flush volumes must be a minimum of 30 μL for injections with air segment, and 35 μL for injections without air segment. If samples are highly viscous it may be necessary to program larger flush volumes and reduce the syringe speed for better performance.



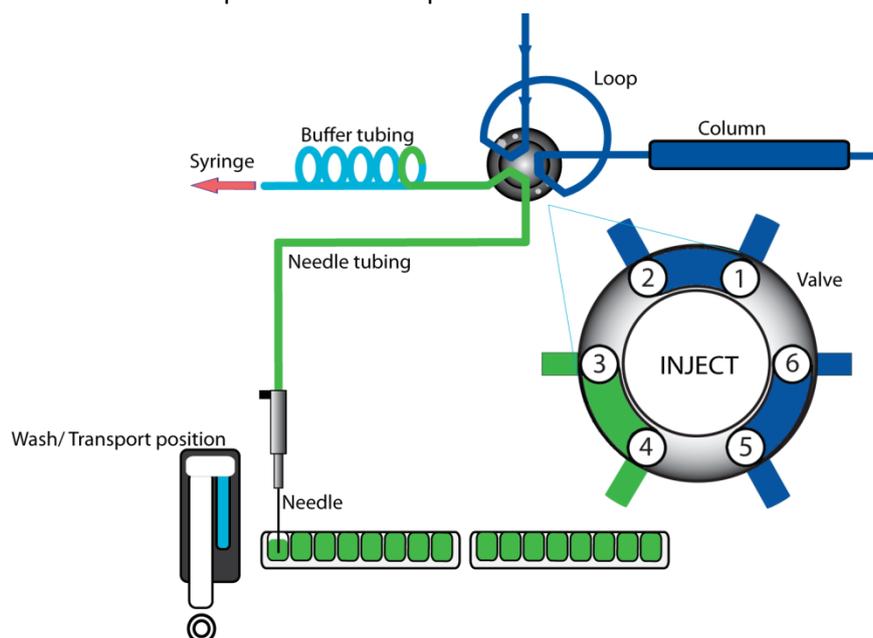
Partial loopfill injections

The switching sequence for a partial loopfill injection is:

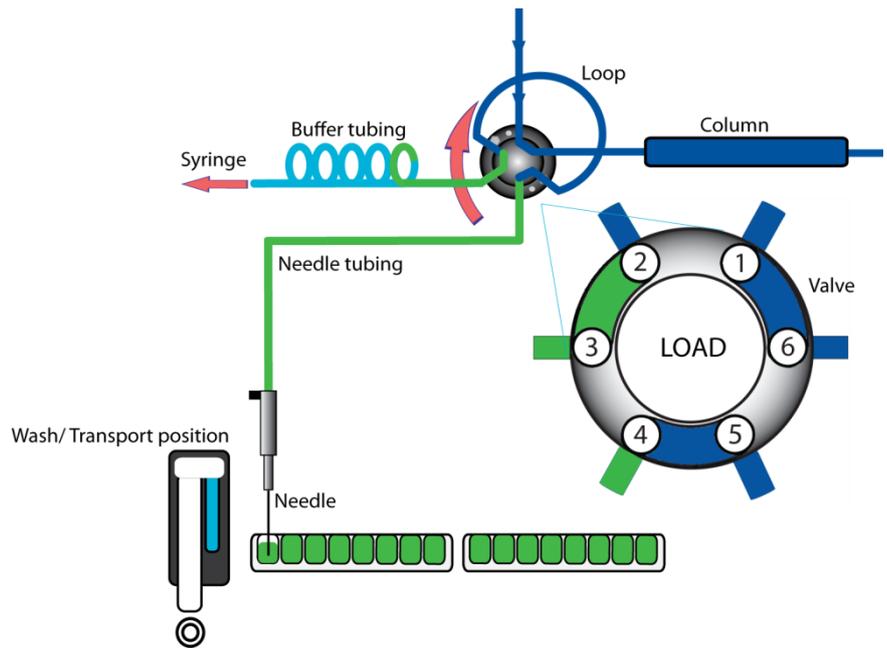
- 1 The initial situation: the injection valve is in the INJECT position. The sample needle with air needle has entered the vial/well. Headspace pressure (optional), applied through the outer air needle, ensures that no air or vapor bubbles are formed during sample aspiration.



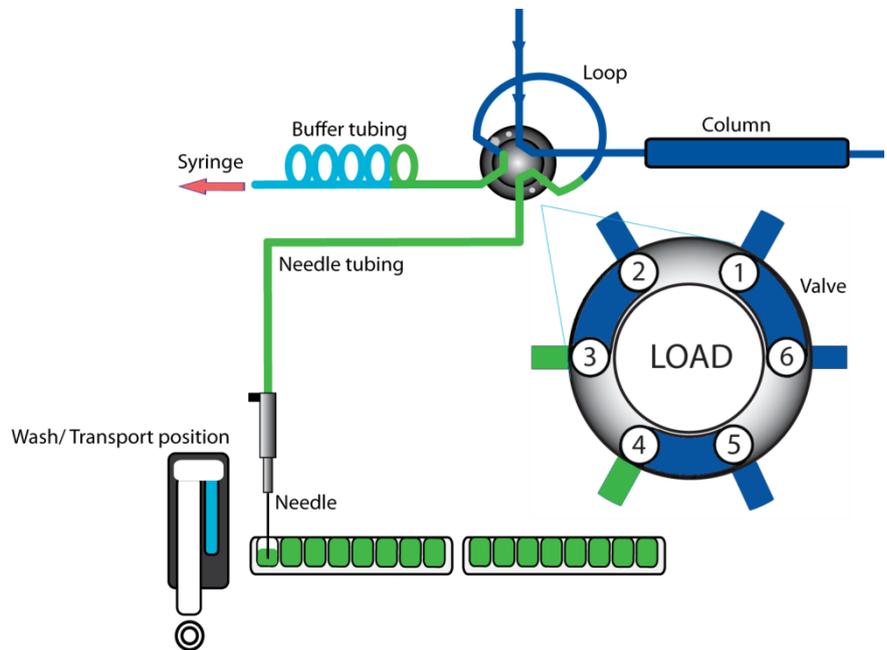
- 2 The syringe dispenser aspirates the "flush volume" from the sample vial to fill the sample line with sample and remove wash solvent.



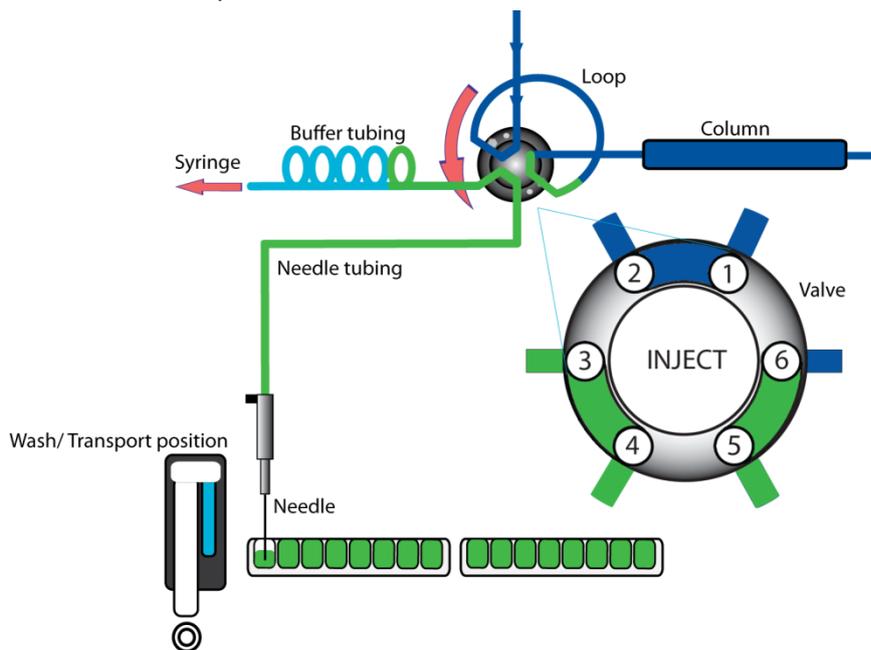
- 3 The injection valve switches to LOAD, ensuring a distinct sample plug at the beginning of the sample loop.



- 4 The programmed injection volume is now aspirated into the sample loop.



- 5 The injection valve switches to INJECT. The sample loop is now part of the (U)HPLC mobile phase flow path: sample is transported to the column. The analysis starts.

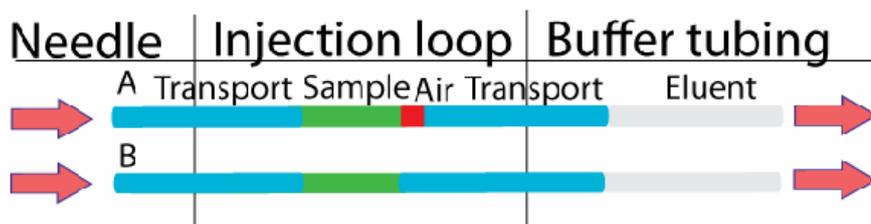


If an injection from the same vial and no wash routine is programmed, the next injection sequence will start with a flush of 50% of the programmed flush volume. Otherwise, it will start with a flush of the programmed flush volume. If the withdrawal of sample for the next injection exceeds the total volume of the sample buffer tubing, the buffer tubing is rinsed before the next injection. The next injection will start with the programmed flush.

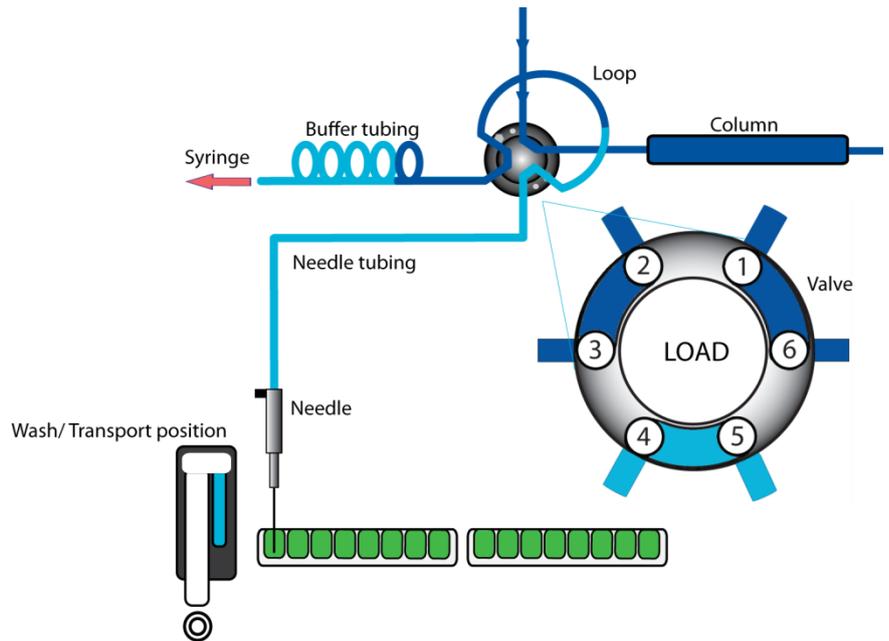
Air segment with Partial loopfill injections

An air segment can be used to reduce the amount of flush volume. The air segment is at the front of the flush volume and will not be injected.

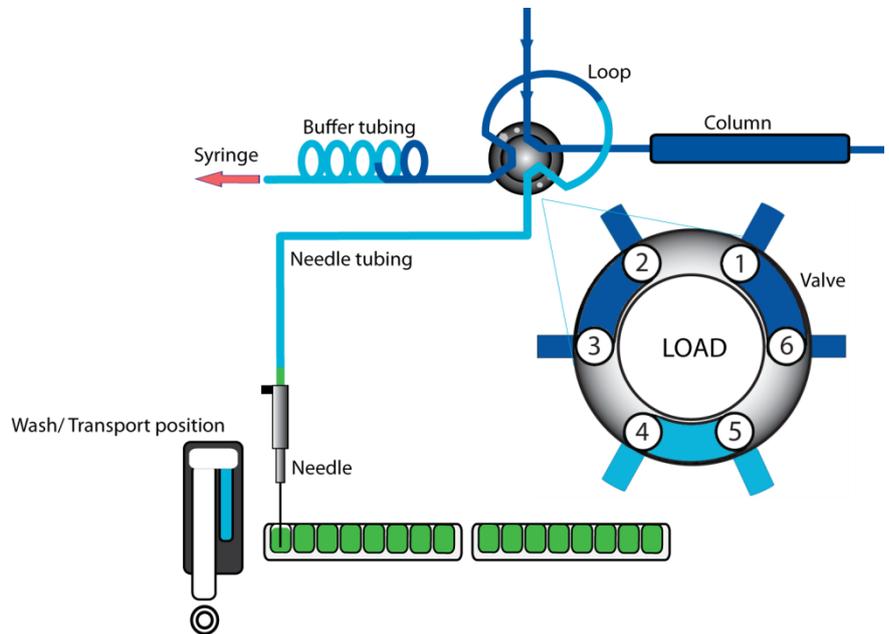
With a standard needle, the flush volumes must be a minimum of 30 μL for injections with air segment and 35 μL for injections without air segment. If the samples are highly viscous, it may be necessary to program larger flush volumes and reduce the syringe speed for better performance.



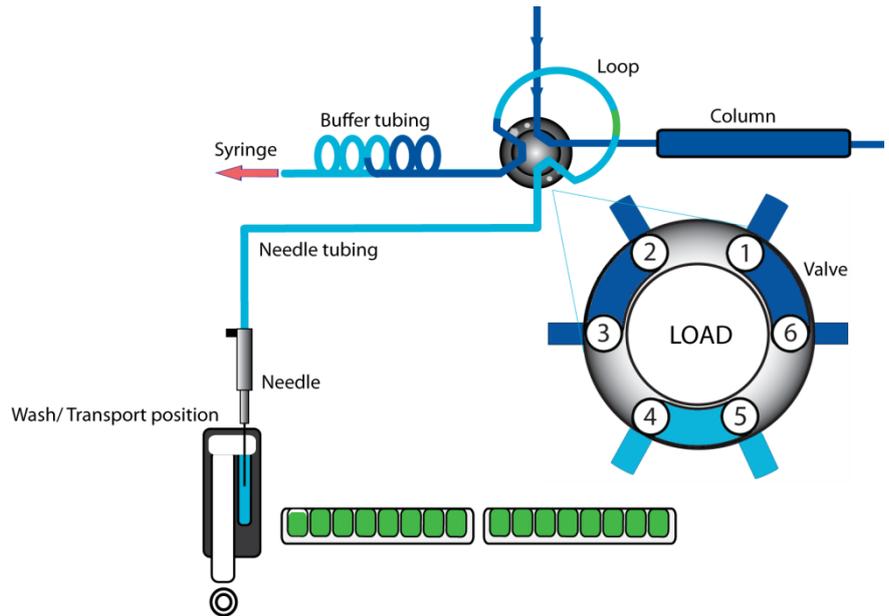
- 4 The needle moves from the transport position to the sample vial.
The injection valve switches to LOAD position.



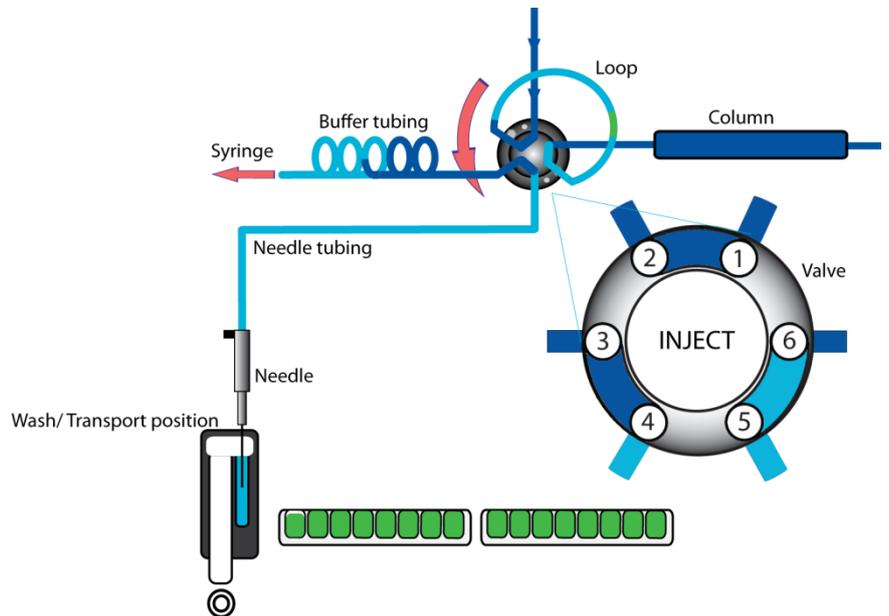
- 5 The programmed injection volume is aspirated from the sample vial.



- 6 The sample needle moves back to the transport position. A second transport plug is aspirated. The sample is quantitatively transported into the loop.



- 7 The injection valve switches to INJECT. The sample loop is now part of the (U)HPLC mobile phase flow path: sample is transported to the column. The analysis timer starts.



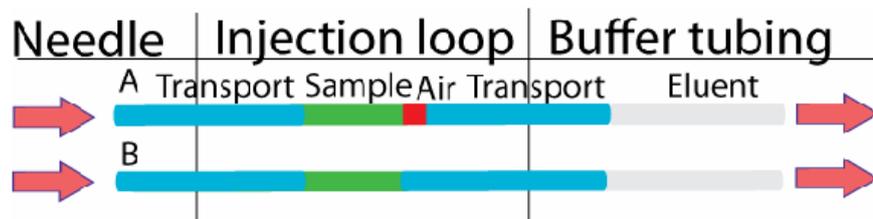
The sequence is repeated for each injection.

Air segment with μL Pickup injections

If an air segment has been programmed, it appears at the front of the first plug of transport liquid and at the front of every sample plug.

In this injection mode:

- the air segment at the front of the sample plug is injected into the (U)HPLC system
- no headspace pressure can be applied on vials/wells in this mode to avoid sample errors due to air expansion during exchange from the sample vial/well to the transport position.



{“Special Considerations”} Special Considerations

Atmospheric conditions should be relatively free of ammonia and carbon dioxide to avoid interference with measurement of amino acids. This is very important when working at high sensitivity.

{“Lifting and Carrying”} Lifting and Carrying

The autosampler is 21kg. No lifting aids are supplied. If the instrument is moved from its location, it must be carefully lifted from the bottom edges at either side. Never lift the instrument from the front as the door may come off the hinges. Disconnect the autosampler mains cable, pipes, and control cables before moving the instrument

{“Device and Waste Disposal”} Device and Waste Disposal

The waste container must be located below the level of the instrument. It should be labelled according to local regulations. The waste produced by the Amino Acid Analyzer is classified as harmful.



Device must be taken to separate collection at the end of their working life. Do not dispose of these products as unsorted municipal waste: take them for recycling. For info on your nearest recycling point, check with your local waste authority.

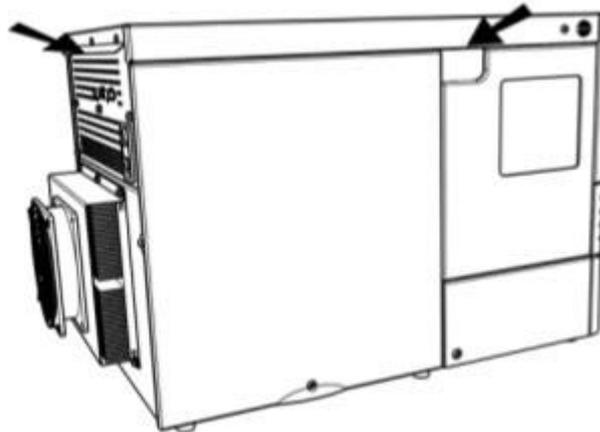
Installation

Check that all items (refer to packing list) are present.

Biochrom Alias autosampler

Execute the following steps for initial installation of the Alias autosampler:

- 1 Lift the Alias from its packaging using both hands at the marked position:



- 2 With both hands *under* the instrument, lift the Alias to its operating location. Keep the instrument upright.



Make sure that the ventilation holes at the back of the autosampler are not blocked. Note that if the ventilation holes are blocked, this may influence performance and cooling capabilities of the autosampler.

If objects are placed on top of the Alias, this may also influence the cooling capabilities.

Objects can be placed on any side of the Alias; however, make sure these objects are placed at a distance of:

- 5 cm from the Alias, if objects are placed at only **one** side of the Alias
- 10 cm from the Alias, if objects are placed on **more than one side** of the Alias

Do not place the Alias in an area subject to excessive dust or shocks. Use the Alias indoors only. Do not place it near a source of heat or in direct sunlight, as this may influence the cooling capabilities of the system.

- 1 Leave the Alias to adopt ambient temperature for at least one hour.
- 2 Install Biochrom Alias Manager on your PC.
- 3 Check that fuses and voltage range on the rear side of the instrument match that for the power outlet to be used.



Use a power supply with protective grounding only.

- 4 Connect the Alias to the PC COM-port with the cable provided with the Alias.
- 5 Connect the power cable between the Alias and the power outlet.
- 6 Switch on the Alias.
- 7 On your PC, open Biochrom Alias Manager and enter the required settings.
- 8 Connect the drain tubing to the waste outlet.
- 9 Fill the wash solvent bottle inside the sampling compartment of the Alias with distilled water and propanol (80/20 v/v%) or mobile phase. Only water or organic solvents should be used. Do not use crystalline or buffer solutions, as these may block the system and cause severe damage. Degas the wash solvent to prevent air bubbles from forming in the syringe.
- 10 Fill the wash solvent tubing, syringe and buffer tubing by washing the system two or three times.

Use 100% IPA for better degassing or removing of air bubbles.

- 11 Check if air bubbles are trapped in the syringe; remove them by gently tapping the syringe.
- 12 Connect your HPLC pump to port 1 of the injection valve and the column (or the capillary) to port 6 of the injection valve. Check for leakage and let the system stabilize for at least 5 minutes.

Biochrom Alias Manager Software

A software package is supplied with the Alias autosampler: the Biochrom Alias Manager. It allows you to perform the following functionality:

- Set the temperature of the cooling sample tray.
- Move the tray home or front for easy access to the samples.
- Set the needle wash volume.
- Set other parameters such as needle height, syringe wash, valve positioning.
- Service manager, accessible by Service Engineers

Installation

The Alias Manager is installed at the same time as Biosys software. Please refer to the ***Software Suite Installation and Setup Guide.pdf*** for guidance on how to install and set up the Alias Manager.



Do not connect the PC to the Internet to protect from malware attacks.

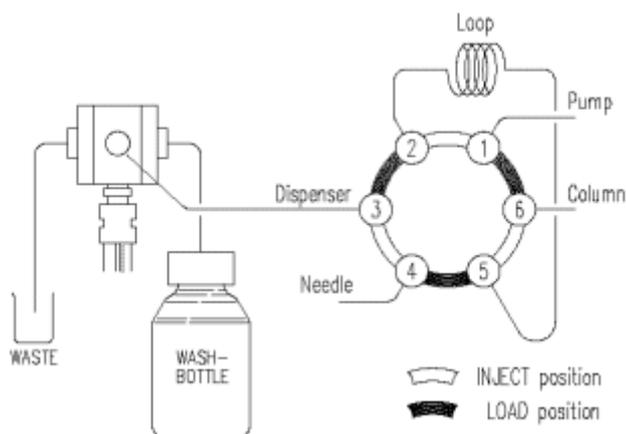


Unauthorized use of Autosampler is forbidden; the instrument and control software can be used only by authorized and trained individuals.

Alias fluid connections

When all items have been installed, the following fluid connections are in place:

Figure 11: Fluid connections



The Alias is factory-installed with:

- 500 μ L syringe
- 200 μ L sample loop
- 1000 μ L buffer tubing
- 15 μ L stainless steel sample needle.



Make sure that the following are correctly connected:

Buffer pump to port 1 of the injection valve.

Column to port 6 of the injection valve.

Alias tubing

The Alias is standard fitted with the following tubing:

Tubing	Materials/Dimensions
Standard sample needle and tubing (label 15 μ L)	SS: 97 mm x 0.8 mm OD x 0.25 mm ID ETFE (Tefzel): 200 mm x 1/16" OD x 0.25 mm ID
HPLC buffer tubing from high-pressure valve to syringe valve (label 1000 μ L for HPLC)	ETFE (Tefzel): 1275 mm x 1/16" OD x 1.0 mm ID
Tubing syringe valve to wash solvent bottle	PTFE: 400 mm x 1/8" OD x 1.6 mm ID
Tubing syringe valve to waste	PTFE: 400 mm x 1/8" OD x 1.6 mm ID

Note the following if you need to install new tubing:

- Insert tube ends always flush with ferrule ends
- Do not over tighten nuts, as this may cause blockage in the flow path
- Make sure that you always use tubing volumes that are suitable for use with the other items in the flow path.

Tubing guide

To prevent that the wash tubing obstructs the horizontal movement of the needle unit, use the tubing guide integrated in the leakage drain:

Figure 12: Tubing guide

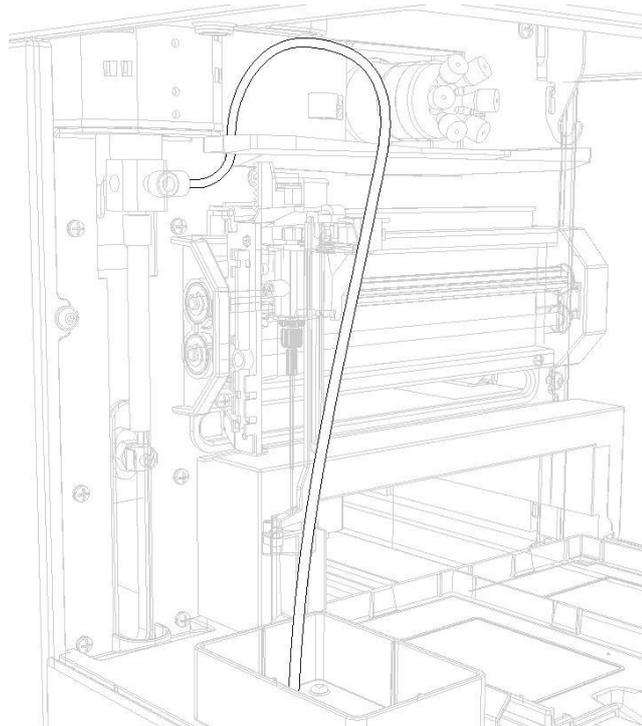
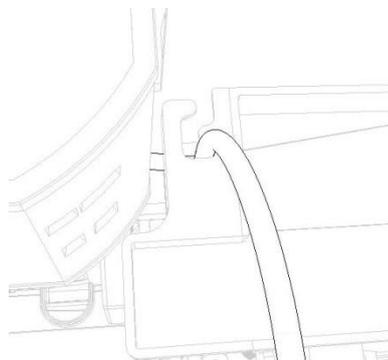


Figure 13: Top view of tubing guide



Waste tubing

Make the following connections for disposal of waste liquids:

- General waste: connect the drain tubing (in the shipkit of the autosampler) to the right-hand drain hose connector (see figure 8, number 2). Place the other end in a bottle for waste (on the floor). Through this drain all the liquid dispensed to the wash position is removed. Sample liquid that is not injected is also removed through this tubing.
- Condensation water and leakage drain: through the left-hand hose connector (see figure 8, number 3) all leaked solvents and condensation (from cooling) are drained. If the cooling is used, you are advised to connect this hose connector (in the shipkit of the autosampler) to a waste container on the floor.

Make sure that none of the drain or waste tubes is twisted; this might obstruct the flow path.

Wash solvent and syringe rinse

Use a clean bottle for the wash solvent and place it on the left-hand side of the Alias. You are recommended to use a mixture of distilled water and isopropanol (80 /20%) as wash solvent. Before using the wash solvent, degas the solvent with helium or an ultrasonic bath. Do not use salts or buffer solutions; crystals may block or damage the system.

To fill the wash solvent tubing execute the following steps:

- 1 Place the end of the wash solvent tubing in the filled wash solvent bottle.
- 2 Go to the settings tab in the Biochrom Alias Manager.
- 3 In the Syringe control box, click **End**. A syringe volume of wash solvent is aspirated from the wash solvent bottle and the wash solvent tubing is filled.
- 4 Click **Home**. The syringe content is dispensed to syringe waste.
- 5 Repeat steps 3 and 4 until the wash solvent tubing and the syringe are completely filled.
- 6 When wash solvent tubing and syringe are completely filled, click **Start** in the wash control box to perform a standard wash routine. All tubing connected to the syringe valve will be rinsed with wash solvent.
- 7 The Alias is initialized.

Syringe

A 500 µL syringe is standard installed in the Alias. However, it is also possible to install a 250 µL or a 2500 µL syringe. Refer to ***Syringe dispenser*** (on page 38) for more information on how to replace the syringe.

Note that the Alias will give the best results if all air is removed from the syringe. Execute an extra wash to remove air from the syringe.

Sample handling

Take the following into account when handling samples:

- Standard vials can best be filled by means of a narrow-end pipette to allow air to escape when filling the vial.
- Do not fill vials to the edge. If you do, sample will be forced into the air needle, risking cross-contamination of samples and soiling the needles.
- It is important that seals and capmats are airtight to prevent air bubbles from forming and to block evaporation of volatile samples. We recommend use of the following seal types:
- for vials: standard septa (thin types); do not use vials with hard caps that are not designed for being pierced by an injection needle (do not use e.g. Eppendorf SafeLock micro test tubes).
 - When you use uncapped vials, injection performance may not be to specification.
 - Filtering the eluent with 0.2 µm filter will considerably reduce the risk of clogging. The same applies for the samples; make sure you use the appropriate filter material for sample filtration.

Maintenance

For all maintenance procedures:

- 1 Open the door of the Alias.
- 2 If the cooling option is installed: remove the cooling cover by sliding it towards you.
- 3 Press the two buttons at the top sides of the Alias simultaneously.
- 4 Remove the cover by pulling it towards you.



You do not need to disconnect the Alias from the power source for any of the maintenance procedures. In this way software control will still be possible.

Cleaning

In general, the Alias autosampler needs very little maintenance. You can clean the outside with a damp cloth with non-aggressive cleaning liquid. Other items that may need periodic cleaning:

- **valve leak bin** (see "Alias autosampler - front" on page 8): a special leak bin is installed underneath the injection valve. You can clean this bin with a damp cloth with non-aggressive cleaning liquid.
- **sample tray**: if sample has been spilled on the sample tray, clean the tray with a damp cloth with non-aggressive cleaning liquid.
- **drain tubing**: regularly flush the drain tubing with solvent to prevent clogging and to ensure that liquids and condensate are disposed of.

Injection valve and rotor seal

The Alias is equipped with an injection valve, either with quick-connect mounting, or with fixed mounting.

Execute the following steps to remove the injection valve:

- 1 Disconnect all tubing from the valve. Only the sample loop can stay in place.
- 2 Remove the valve.
- 3 Remove the screws from the stator part of the valve.
- 4 Gently open the valve and take out the rotor seal. Clean and/or replace the seal.
- 5 Place the stator back on the rotor and fasten the screws.
- 6 Hold the valve for mounting with port 1 pointing upward.
- 7 Place the valve into its slot and fasten it.
- 8 Reconnect all tubing to the valve.
- 9 Perform a standard wash.

The Alias is now ready for use.

Sample loop

The Alias is standard fitted with a 200 µL sample loop, A different sample loop size can be installed, but note that you will need the proper combination of syringe and tubing to ensure good results.

Take the following into account when you have installed a sample loop:

- connect the loop between ports 2 and 5 of the injection valve
- go to the service tab of the alias Manager and adapt settings in the loop volume box if you have installed a loop with a different volume.



Remember that the maximum injection volumes are calculated with the following formulas:

full loop: injection volume = loop volume

partial loopfill: maximum injection volume = 50% of the loop volume

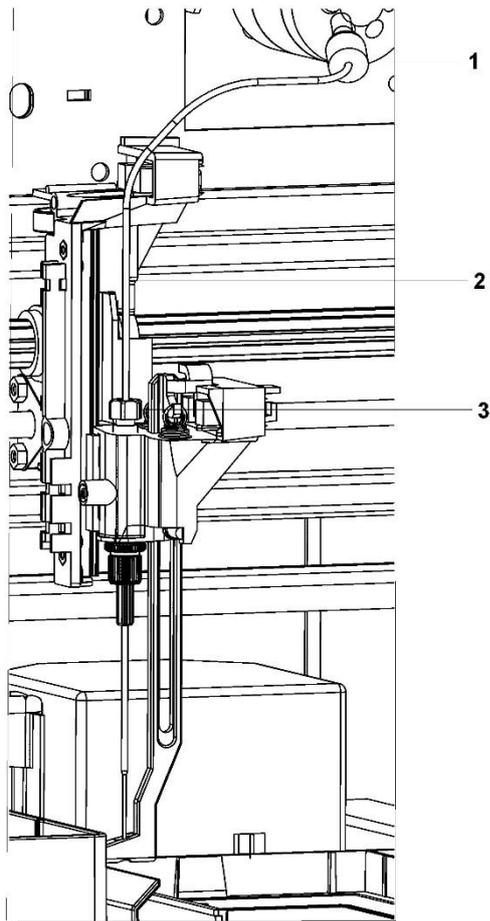
µL pickup: maximum injection volume = (loop volume - 3x needle volume)/2

Replacing the sample needle

Execute the following steps to replace the sample needle:

- 1** Open the Biochrom Alias Manager (BAM).
- 2** Click **needle Exchange** in the Settings tab. The needle moves to exchange position.
- 3** Loosen the needle connection nut (number 3).
- 4** Loosen the nut (number 1) that connects the tubing (number 2) to port 4 of the injection valve.
- 5** Remove the sample needle by pulling it out of its fitting by the tubing.
- 6** Install a new needle assembly; make sure that the air seal is around the needle.
- 7** Tighten the needle assembly with the needle connection nut.
- 8** Connect the other end of the needle connection tubing to port 4 of the injection valve. Do not tighten too much as this may block the tubing.
- 9** Click **wash** in the operations tab of the BAM. The sample needle moves back to home position and a wash is performed.
- 10** Use the needle height option in the settings tab to adapt the Needle height.

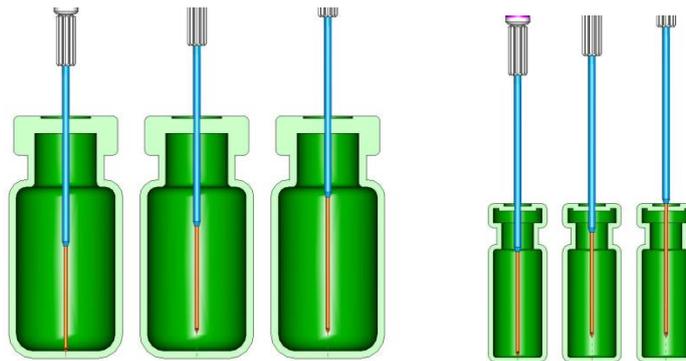
Figure 14: Replacing the sample needle



Air needles for Alias

Standard air Needle

The standard air needle is a 62 mm needle (no. 0045.505). See the illustrations below for the puncturing depth of the needle:



10 mL vial

2 mL vial

Air needle replacement

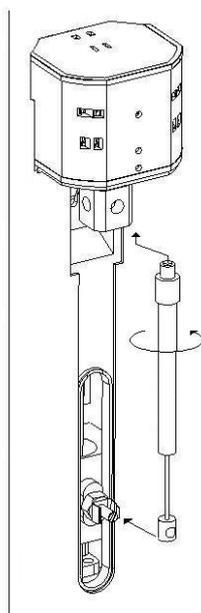
Execute the following steps to replace the air needle:

- 1 Remove the sample needle (Replacing the sample needle).
- 2 Unscrew the chrome locking nut to remove the air needle.
- 3 Unscrew the chrome locking nut from the adjustment nut.
- 4 Screw the height adjustment nut to the chrome locking nut (thread of the height adjustment nut must be level with the lower part of the locking nut). Make sure the O-ring seal is in the locking nut.
- 5 Install the air needle.
- 6 Install the sample needle.
- 7 Program the proper needle height for the new needle in the BAM settings window.
- 8 Perform an initial wash to rinse the needle.

Syringe dispenser

The Alias is standard supplied with a 500 μ L syringe.

Figure 15: Syringe



Execute the following steps to install a different syringe:

- 1 In settings tab of the BAM, click **Exchange** in the Syringe control box.
- 2 Unscrew the syringe from syringe valve, but make sure that the connector in the valve remains in place.
- 3 Disconnect the plunger from the syringe drive.

- 4 Fill the new syringe with wash solvent, preferably IPA (isopropanol). Make sure that most air bubbles are removed from the syringe.
- 5 Connect the plunger of the filled syringe to the syringe drive and connect the syringe with the connector at the syringe valve.
- 6 Screw the syringe firmly into the connector.
- 7 In the BAM, click **Home** in Syringe control box. The syringe moves to home position and its content will be dispensed to syringe waste.
- 8 If there is still some air in the syringe, click **Fill** in the BAM control. The syringe is filled with wash solvent. Use IPA.
- 9 Click **Home** again to dispense the wash solvent to waste.

If there is still air in the syringe, repeat steps 8 and gently tap the syringe as the wash solvent is filled into the syringe.

Perform a standard wash routine. All tubing connected to the syringe valve will be refilled and flushed.

Syringe plunger & plunger tip

Execute the following steps to replace the plunger or plunger tip:

- 1 In the BAM, click **Exchange** in the Syringe control box.
- 2 Remove the syringe (see Syringe dispenser Alias).
- 3 Slide the plunger out of the glass part of the syringe.
- 4 With pliers: remove the tip carefully and make sure you do not damage the stainless steel plunger.
- 5 Dampen the new tip with for example isopropanol.
- 6 Press the new tip on the plunger.
- 7 Insert the plunger in the glass part the syringe.
- 8 Install the syringe (see Syringe dispenser) in the autosampler again.

Replacing the Syringe dispenser valve

The syringe valve is a 4-port selection valve. Ports are assigned as follows:

Waste	Use this port as a drain for the syringe dispenser.
Wash	Use this port to aspirate wash liquid from the wash bottle (or in case of multiple wash liquids: connect it to the solvent selection valve)

- Needle Connect the buffer tubing to this port
- 4th port Optional syringe valve port

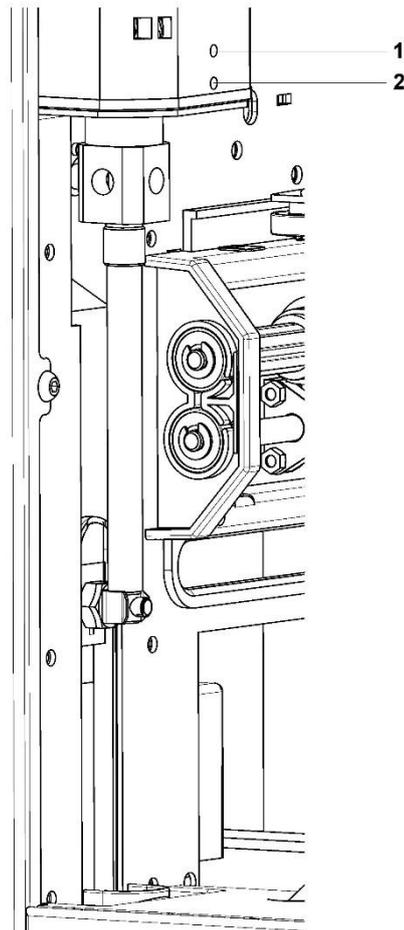
All connections to the syringe valve must be made using fingertight fittings. An exception can be made for the waste outlet (the port on the rear of the valve).

Execute the following steps to replace the syringe dispenser valve:



Place the syringe valve in 4th optional port position before you replace the syringe valve. In this position, the mounting screws are opposite/in line with the holes.

Figure 16: Replacing the syringe dispenser valve



- 1** Move the syringe to exchange position by clicking **Exchange in the Syringe control box** of the BAM.
- 2** Loosen the lower socket-head screw (number 2) a full rotation counterclockwise.
- 3** Loosen the upper socket head screw (number 1) a full rotation counterclockwise.
- 4** Remove the syringe.
- 5** Remove the syringe valve and install a new one. Make sure the flat side of the axle faces forward and make sure that the valve is completely pushed upward.

- 6 Install the new seal.
- 7 Fasten the two socket-head screws again (fingertight + 1/4 turn).

Fuses

Fuses of the following types are installed in the autosampler:

2 x 2.5 AT



Disconnect the autosampler from its power source if you need to replace fuses.

If you need to replace the fuses, make sure that you install fuses of the same type and rating.

Fuses are in the fusebox at the back of the autosampler.



Contact Service if problems with fuses are recurring.

Trouble shooting

Even though great care was taken in the design of the Alias, problems may occur:

- **instrument errors** (on page 42): these can be caused by a variety of reasons.
- **software errors** (on page 43): usually caused by faulty communication between instruments, or by faulty installation of the software.
- **analytical problems** (see "Analytical trouble shooting" on page 43): these may occur e.g. as a result of wear of parts, errors in injection settings and methods, or a wrong combination of sample loop, buffer tubing and syringe.

Biochrom Alias Manager contains a Service option (select Service tab). Note that an access code is required for this option, and that the service option is intended for service engineers only.

Contact your supplier if a problem occurs that you cannot solve.

Instrument errors

Incidental fault conditions may occur in any instrument. The Alias will generate an instrument error message with an error number, a short description of the error and instructions on how to proceed.

In most cases, you will be asked to either initialize the system, or to switch the system off and then on again. Always click **OK** and follow the instructions to resolve the error status.



Make sure Alias is connected to a grounded power source.

If the LED is not lighted, a fuse may have blown.

Checking a valve implies that you remove the valve and check all parts for wear and dirt. Execute the following steps after any problem with a valve has been resolved:

- 1 In the Initial wash group box of the BAM, click **Start** to start the wash.
- 2 Click **Stop** to end the wash.

Execute the following steps if you are asked to switch the system off, and then on again:

- 1 Check that the communication cable between Alias and PC is properly installed.

- 2 Turn the instrument off with the on/off switch at the back of the autosampler.
- 3 Turn the system on again with the on/off switch. The system is initialized and is now ready for use.

Software errors

Software errors usually are caused by faulty installation of the software, or by faulty communication between instruments; you will be asked to re-install the software on the PC that controls the system.

If a software error message appears, first check if it may be caused by faulty communication between instruments.

Analytical trouble shooting

Analytical problems like bad reproducibility or carry-over may occur in any HPLC system. It may be hard to find the cause; you may have to try out several procedures. The first thing to do is to determine whether the problem is caused by the autosampler or by the rest of the system:

- 1 Replace the valve by a manual injection valve to discriminate between valve problems and other problems.
- 2 Do a number of Full loop injections. If the results are fine, the fault is in the autosampler; if not, check the rest of the HPLC system.

Please bear in mind that analytical problems may also be caused by external influences like temperature or light-sensitive samples. Make sure that the application was running trouble-free before and that no changes have been made to the system.

A number of causes and possible solutions for analytical problems is listed below. Contact service if you need further help.

If **reproducibility** is not according to specifications, check the following possible causes:

Causes:	Solutions:
Air in flow path.	Do an initial wash (select wash in the Biochrom Alias Manager)
Leaking syringe.	If leakage occurs at the top of the syringe, check whether it has been properly mounted. If leakage occurs at the bottom of the syringe, replace plunger tip or syringe.

Leaking syringe valve.	Check or replace valve.
Rotor seal worn out.	Replace seal. Check stator.
Dead volumes in tubing connections.	Redo connections with new ferrules and nuts.

If a **blank gives a peak that is too high** for your criteria:

<u>Causes:</u>	<u>Solutions:</u>
Solubility problem.	You can either modify your sample, or accept carry-over.
Bad match between sample characteristics and hardware.	<p>Check hardware:</p> <p>Needle: either use an extra wash (to wash the inside and outside needle), or install a different type of needle (Steel or Silica-coated)</p> <p>Valve: replace rotor in valve by Valco E or H type.</p> <p>Tubing: install different tubing (Steel, Peek) between autosampler and column, or use different wash solvents</p>
The blank you use has been soiled.	Use a new blank.
Cause not clear.	Check if you can solve the problem by using more variation in solvents.

If **no injection** takes place:

Causes:	Solutions:
Blockage in flow path	<ol style="list-style-type: none">1 Disconnect needle from valve.2 Start a manual wash.3 If solvent flows from the injection port, check the needle; if no solvent flows from the injection port, disconnect buffer tubing from valve.4 Start a manual wash.5 If solvent flows from open end: check rotor seal; if not: disconnect buffer tubing from syringe valve.6 Start a manual wash.7 If solvent flows from syringe valve: check buffer tubing; if not, check for over-tightened connections in the entire flow path and check the syringe valve.
Leakage in the injection valve	<ol style="list-style-type: none">1 Disconnect the needle tubing and buffer tubing.2 Connect port 1 to an HPLC pump.3 Block port 6.4 Start the pump at a low flow.5 Observe ports 3 and 4 for leakage.6 If leakage occurs at ports 3 and 4: check rotor seal; if not: recheck with manual valve.
	<hr/> <p> Observe the maximum allowed pressure of 350 bar to prevent leakage in the valve (HPLC)!</p> <hr/>

Specifications

General:

Sound pressure level	LeAq < 70 dB
Working temperature	10 - 40°C (indoor use only)
Storage temperature	-25 - +60°C
Humidity	20 - 80% RH
Safety and EMC compatibility	According to EC-directives; CSA (UL) approved
Installation class	II
Pollution degree	2
Altitude	up to 2000 m
Dimensions	300 mm x 510 mm x 360 mm (without cooling option) 300 mm x 575 mm x 360 mm (with cooling option)
Weight	19 kg (without cooling) 21 kg (with cooling)
Max. weight that can be placed on top of Alias	65 kg
Power requirements	95 - 240 Volt AC \pm 10%; 50 - 60 Hz; 200VA
Personal Computer	<ul style="list-style-type: none"> • Intel Dual Core, 3GHz (or equivalent processor) • 4 Gb RAM • 200Gb Hard Disk Drive • Two serial ports. (USB-serial converters not acceptable) • LAN card • Mouse, Keyboard & Monitor • Windows 7 Professional 64-bit. • Microsoft .NET framework v3.5 or higher
Viscosity range	0.1 - 5 cP

Sampling:

Sample capacity	Biochrom 84 positions sample tray with 3 10ml vial holders.
Vial/Plate dimensions (incl. cap)	Max. plate/vial height: 47 mm (incl. septa or capmat)

Loop volume	1 - 200µL programmable, other loop dimensions optional
Dispenser syringe	500 µL standard
Vial detection	Missing vial plate detection by sensor
Headspace pressure	Built-in compressor, but only for vials with septa
Switching time injection valve	Electrically < 100 msec
Piercing precision needle	± 0.6mm
Wash solvent	Integrated wash solvent bottle
Wetted parts in flow path	PTFE, TEFZEL, VESPEL, Glass, Teflon.
Injection cycle time	< 60 sec. in all injection modes for 1 injection ± 100 µL including 250 µL wash (speed setting normal)

Analytical performance:

Injection modes	Full loop, partial loopfill and µL pickup mode, PASA™ (pressure-assisted sample aspiration)
Reproducibility (valid at 1.0 cP)	RSD ± 0.3% for full loop injections RSD ± 0.5% for partial loopfill injections, injection volumes > 5 µL at constant pressure RSD ± 1.0% for µL pickup injections, injection volumes > 5 µL
Memory effect	< 0.05% with programmable needle wash

Programming:

Interface	via Biochrom Alias Manager software
Injection methods	Full loop, partial loopfill and µL pickup
Injection volume	0 µL - 200 µL (with 1 µL increment), depending on system settings
Max. injection volume	<ul style="list-style-type: none"> ▪ Full loop = loop volume ▪ Partial loopfill = ½ of loop volume ▪ µL Pick up = (loop volume - 3 x needle volume)/2
Injections per vial	max. 9 injections
Analysis time	max. 9 hr, 59 min, 59 sec
Wash	Programmable: Wash between injections and Wash between vials

Communication:

Outputs	preset
Inputs	preset
Serial communication port	RS232C

Options (factory installed):

Sample tray cooling	Built-in Peltier cooling Range: 4°C to Ambient - 3°C Temp: air temperature in sample compartment: 4°C ± 2°C (at temperature sensor) (Temperature at relative humidity of 80% and ambient temperature of 25°C)
Bio-compatible sample flow path and valve	Inert sample needle (Silco steel) and bio-compatible valve (PEEK)

Control I/O connections

The autosampler has two RS232 connections which are preconfigured:

- RS232 connector for serial communication
- RS232 connector for I/O signal



The manufacturer will not accept any liability for damages directly or indirectly caused by connecting this machine to instruments which do not meet relevant safety standard.

List of errors

The following errors may occur with Alias:

Tray unit

- ERROR 294 Home sensor not reached.
- ERROR 295 Deviation of more than +/-2mm towards home.
- ERROR 296 Home sensor not de-activated.
- ERROR 297 Home sensor activated when not expected.
- ERROR 298 Tray position is unknown.

Needle unit

- ERROR 303 Horizontal: needle position is unknown.
- ERROR 304 Horizontal: home sensor not reached.
- ERROR 306 Horizontal: home sensor not de-activated.
- ERROR 307 Horizontal: home sensor activated when not expected.
- ERROR 312 Vertical: needle position is unknown.
- ERROR 313 Vertical: home sensor not reached.
- ERROR 315 Vertical: home sensor not de-activated.
- ERROR 317 Vertical: stripper did not detect plate (or wash/waste).
- ERROR 318 Vertical: stripper stuck.
- ERROR 319 Vertical: The sample needle arm is at an invalid position.

Syringe dispenser unit

- ERROR 324 Syringe valve did not find destination position.
- ERROR 330 Syringe home sensor not reached.
- ERROR 331 Syringe home sensor not de-activated.
- ERROR 334 Syringe position is unknown.
- ERROR 335 Syringe rotation error.

Injection valve

- ERROR 340 Destination position not reached.
- ERROR 341 Wear-out limit reached.
- ERROR 342 Illegal sensor readout.

Cooling unit

- ERROR 347 Temperature above 48°C at cooling ON.

Electronics

ERROR 280	EEPROM write error.
ERROR 282	EEPROM error in settings.
ERROR 283	EEPROM error in adjustments.
ERROR 284	EEPROM error in log counter.
ERROR 290	Error occurred during initialization, the Alias cannot start.

List of accessories and spares

The standard shipping kit for the Biochrom Alias contains:

- a shipping kit list
- a silicon drain hose
- shipping kit for the injection valve (Valco, Rheodyne or other)
- 2 x 2.5A TL 250V fuse for power connection
- Wash bottle
- CD with user manual
- Needle for high titre plates (needle for low titre plates is in instrument)
- IO cable
- serial cable

List of spare parts:

Qty	Description	Biochrom P/N
1	Air needle 62mm Natural	80-6001-60
1	Vial tray 84*2 mL + 3* 10mL	80-6001-91
1	Plunger replacement tip 500 µL each	80-6001-59
1	Injection valve module	80-6001-88
1	Needle module	80-6001-63
1	Tray drive module	80-6001-90
1	Sample needle Bio with Valco connections	80-6001-61
1	Piggy back cooler Bd	80-6001-89
1	Wash position incl. screws	80-6001-67
1	Syringe valve including valve bush	80-6001-73
1	Buffer tubing 1000µL Tefzel with Valco connections	80-6001-68
1	Wash bottle 250 mL rectangular	80-6001-62
1	Dispenser module	80-6001-87
1	Cooler module	80-6001-86
1	Biochrom main board configured	80-6001-64
1	Power supply 200W-24V	80-6001-71
1	Optical sensor TLP1253	80-6001-85
1	Stepper motor KH42HM2B155 (Tray drive)	80-6001-65
1	Stepper motor KH56JM2U078 (Syringe drive spindle)	80-6001-66
1	Stepper motor KH56JM2U079 (Syringe drive valve)	80-6001-79
1	Stepper motor KH56JM24080 (Needle drive)	80-6001-78
1	Fuse 2.5 A	80-2118-46
1	Rotor seal for Peek valve C2-2346	80-2111-16
1	Stator for Peek valve C2-2346	80-2111-17
1	Syringe 500µL	80-6001-58
1	Ethernet Cable CPU - Autosampler	80-6001-51

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