

Hoorn 131 2404 HH Alphen a/d Rijn The Netherlands

AS 110 autosampler

User manual

191.0010, Edition 13, 2023





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We Antec Scientific, Alphen a/d Rijn, The Netherlands, declare that the product:

to which this declaration relates, is in conformity with the following CE directives:

Machinery Directive (2006/42/EC)

Low Voltage Directive (2014/35/EU) applied with the following standards:

Safety requirements for electrical equipment for measurement, control, and laboratory use:

 Part 1: General Requirements
 Part 2-081: Particular requirements for automatic
 and semi-automatic laboratory equipment for analysis and other purposes



EMC Directive (2014/30/EU) applied with the following standards:

Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General Requirements EN 61326-1:2006

RoHS directive (2011/65/EU) Restriction of Hazardous Substances **WEEE directive (2012/19/EC)** Waste Electrical and Electronic Equipment



Use manufacturer-supplied cable(s) only to connect all I/O's with other devices. Thoroughly connect the shielding to common. Manufacturer will not accept any liability for damage, direct or indirect, caused by connecting/integration this instrument to devices or systems, which do not meet the essential requirements or relevant safety standards.

Alphen a/d Rijn, The Netherlands, April 22, 2022 Dr. N.J. Reinhoud (managing Director)

that

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AS 110 auto sampler type 191

to which this declaration relates, is in conformity with the following UK Statutory Instruments including their amendments and meets the essential requirements of the listed standards:

2016 No 1101
The Electrical Equipment Safety Regulations 2016
 EN 61010-1:2010, IEC61010-1:2010/AMD1:2016
 EN 61010-2-081:2019
2016 No 1091
The Electromagnetic Compatibility Regulations 2016
 EN 61326-1:2013
 EN 61326-2-6:2013
• EN 61000-3-2:2014
 EN 61000-3-3:2013
 EN 61000-4-2:2009
 EN 61000-4-3:2006, A1:2008, A2:2010
• EN 61000-4-4:2012
 EN 61000-4-5:2014
 EN 61000-4-6:2014
 EN 61000-4-8:2010
 EN 61000-4-11:2004
 EN 55011:2009, A1:2010, Class B, Group 1
2012 No 3032
The Restriction of the Use of Hazardous Substances in Electrical and Electronic Equipment Regulations
 EN IEC 63000:2018



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Alphen a/d Rijn, The Netherlands, June 26, 2023 Dr. N.J. Reinhoud (managing Director)

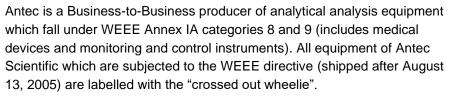
that

Intended use



For <u>research purposes only</u>. While examples of clinical applications may be shown, this instrument is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.

WEEE directive



The symbol on the product indicates that the product <u>must not</u> be disposed as unsorted municipality waste. When taking the instrument out of service, the different materials must be separated and recycled according to national and local environmental regulations.

<u>Collection & recycling information (business-to-business)</u> Antec Scientific offers the possibility for disposal and recycling of their instrument at an appropriate recycling facility if requested (there may be costs involved with this service). Please contact Antec for more information about this service and to register the return and disposal of end-of-life instruments (<u>support@antecscientific.com</u>). To assure hygienic & personal safety <u>all</u> instrument should be returned with a signed decontamination form which is available on the website.

Shipping address for end-of-life products:

Antec Scientific Hoorn 131 2404 HH Alphen a/d Rijn, The Netherlands

In case of questions, or if further information is required about the collection & recycling procedure, please contact Antec or your local distributor.



ROHS directive

The AS 110 is ROHS compliant and in conformity with Directive 2011/65/EC Restricted use of Hazardous Substances in electrical and electronic Equipment (ROHS).



Antec Scientific is an ISO 9001:2008 certified company.

About this manual

This guide is written for laboratory technicians who use the AS 110 autosampler for execution of analytical runs.

An index allows the user to find required information quickly.

Symbols

The following symbols 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 biohazard sign draws attention to the fact that use of biological materials, viral samples and needles may carry a significant health risk.



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 instructions

Adhere to the following standard quality control procedures and the following equipment guidelines when using the auto sampler. The following safety practices will ensure safe operation of the auto sampler and should only be executed by authorized service personnel:

Working environment & safety



The intended use of the system is to introduce sample into a mass spectrometer or other (U) HPLC detection system in a GLP-approved environment. Operators using the system should have the appropriate education an extensive understanding of GLP rules and be skilled in the art. Use this system ONLY for the intended use. Use of the system for any other purpose will cause unsafe situations.

System Operation



To keep up the high performance of the autosampler we recommend that the system is checked regularly and maintenance procedures are carried out. Preventive maintenance contracts are available for that purpose. Please contact your local dealer or the nearest sales office for more information.

Electrical safety



Removal of panels may expose users to dangerous voltages. For that reason this should only be done by authorized service engineers. Disconnect the autosampler from all power sources before removing protective panels. Replace or repair faulty insulation on power cords immediately after discovery of the fault. Check that the actual power voltage is the same as the voltage for which the instruments are wired. Make sure power cords are connected to correct voltage sources. Replace blown fuses with the size and rating indicated on the fuse panel and as listed in the list of accessories and spares (appendix D) of this manual. The Alias must only be used with appliances and power sources with proper protective grounding to prevent damage through build-up of static electricity.

Solvents



The solvents used may be flammable and toxic. The room in which the system is installed should be well ventilated to prevent that solvent vapors cause poisoning or ignite and cause a fire. Use of open fire in the vicinity of this system must be strictly prohibited. Do not install the system in the same room with any other equipment that emits or could potentially emit sparks.

Provide protective equipment near the instrument. solvent gets into the eyes or on the skin, it must be flushed away immediately. Provide equipment, such eye wash stations and safety showers, as close to system as possible. Sample containers (vials) should be sealed to minimize any risks related to solvent vapor. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable and/ toxic solvents through the municipal sewage system. Perform periodic leak checks on supply lines.

Biological Hazard



When you analyze biological fluids you need possible precautions and treat all specimens potentially infectious. Always wear protective and gloves when handling toxic or biologically infectious samples to prevent bio hazards or hazards while working with the Alias.



Waste disposal

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



Applications: quality control

It is recommended that you routinely run several quality control samples. Quality control samples should represent low, average and high levels of a compound. Make sure that quality control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality control samples are out of range may not be valid. Do not report this data until you are certain that system performance is acceptable. Apart from use of quality control samples, we recommend that you use blanks. The blanks will help you assess whether carry-over is within an acceptable range and monitor the integrity of your data.



Using the AS 110 in other ways than indicated in the manual or defined by good laboratory practice may result in unsafe operation.

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CHAPTER 1

Introduction

The AS 110 standard and AS 110 micro autosamplers are high throughput autosamplers 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 AS 110 is designed for indoor use. The AS 110 autosampler features among other things:

- PASA[™] injection concept (see page 10).
- High-resolution syringe control; this ensures very high precision for injection and reagent addition.
- Internal standard addition, sample dilution or derivatization can simply be programmed.
- PC control ensures easy-to-understand operation; contextsensitive online help is available with every window and dialog.
- Special attention has been paid to ensure a service-friendly design.
- To enhance safety, speed of operation of the AS 110 will decrease when the door is opened.
- Optional sample cooling ensures consistent results.

Read this chapter to help identify parts of the AS 110 auto sampler, and to learn more about injection principles.



Autosampler configurations

There are differently configured AS 110 autosampler with the details listed in Table 1.

All replaceable parts are easily accessible. Refer to the List of accessories and spares (Appendix I) for more information.

Table 1.Different AS 110 autosampler configurations, with details of installed parts. Abbreviations: St = standard; m=micro; 6p/10p=6/10 port; U=UHPLC. *) L versions have a LAN connection for remote control instead of RS232 (LAN version are introduced in 2015).

Part number	Type	Cooling option	Sampling valve	Valve port size (inch)	Sample needle (uL)	Syringe (uL)	Buffer tubing (uL)	Sample loop (uL)
191.0035(L*)	St	+	6р	1/16	15	500	1000	100
191.0035U(L)	St	+	6p U	1/32	15	250	200	5
191.0036(L)	St	-	6р	1/16	15	500	1000	100
191.0037(L)	m	+	6р	1/16	2.4	25	200	20
191.0037U(L)	m	+	6p U	1/32	2.4	25	50	5
191.0038(L)	m	+	10p	1/16	2.4	25	200	2 (2x)
191.0038U(L)	m	+	10p U	1/32	2.4	25	50	2 (2x)
191.0041(L)	St	+	10p	1/16	15	500	1000	5 (2x)
191.0041U(L)	St	+	10p U	1/32	15	250	200	5 (2x)

Instrument description

AS 110 autosampler – front

The AS 110 sampling compartment houses the following parts:

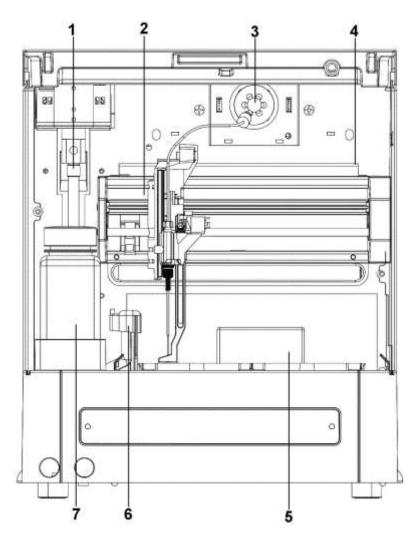


Figure 1: AS 110 sampling compartment.

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

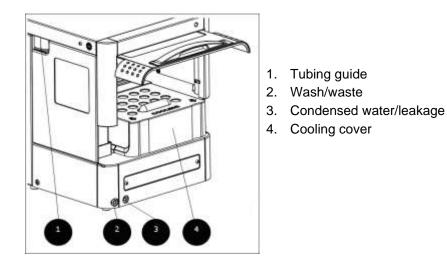


Figure 2: AS 110 with cooling, front-side.

AS 110 autosampler – back

The back of the autosampler has the following items:

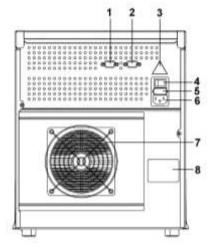


Figure 3: AS 110 with cooling, back-side.

- 1. 9-pin male connector (inputs/output)
- 2. 9-pin female connector (serial interface) or LAN connector (models from 2015 onwards, can be recognized by the 'L' in part number)
- 3. warning label (see "I/O connections" on page 79)
- 4. on/off switch
- 5. fuse box
- 6. power connector
- 7. cooling fan (if cooling option is installed; do not obstruct!)
- 8. type label

Access

To open the door:

- 1. Get hold of the door handle
- 2. Gently pull it towards you and push it upward until it is in horizontal position.
- 3. Slide the door into the autosampler.

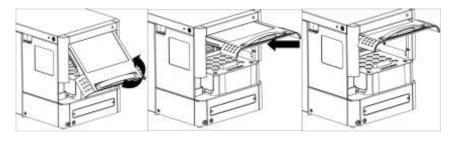


Figure 4: Opening the door.

For easier access to e.g. the syringe, the whole front cover of the AS 110 can be removed (figure 4). To remove the cover:

- 1. Press the two black buttons on either side (top) of the autosampler simultaneously.
- 2. Gently pull the cover towards you.

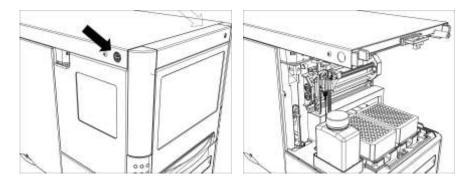


Figure 5: Removing the AS 110 front cover. The black arrows indicate the location of black push buttons. Right picture shows the AS110 without the front panel.

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

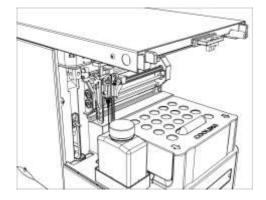


Figure 6: AS 110 with cooling cover

Sample trays

The sampling compartment of the AS 110 can accommodate two sampling plates. Standard high or low well plates or vial trays (12, 48, 96 and 384 positions) can be used. Any combination of well plates is allowed, except for 384 Low on the left and 96 High on the right

A set of two stainless steel 48-positions sample stays are standard supplied with the AS 110 autosampler.

Small samples

For the sampling of small samples (<20 uL), we recommend to use the following combination of vials and trays:

Vials/caps:

The vials/caps combination that fit in the holder shown in Figure 8 are:

- Sample Vials polypropylene 300 uL (Microbiotech, pn. 4001048)
- 8 mm Crimp cap with PTFE seal for single use (Chromacol, pn. 8-ACT)

NOTE: There are subtly different shapes of fraction collector vials on the market. The types that fit best in the holder shown in Figure 8 are the ones from Microbiotech, depicted on the left side in Figure 7. The types depicted on the right in this figure are slightly too wide to fit well in the 96 position tray, but such vials can be sampled by placing them in adaptors that fit in the 48 positions tray. Adaptors can be purchased at Antec (pn. 181.0726; Microdialysis coll. vial adapters, 100pcs).



Figure 7. Picture of two slightly different fraction collector vials.

Tray

Form a stabile holder for narrow fraction collector vials by inserting the following two plates (Figure 8) into each other:

- transparent 96-positions plate (Greiner bio-one, pn. 652280)
- 96-positions flat bottom plate (Greiner bio-one, pn. 655101)

A set for four plates for one autosampler can also be ordered at Antec ('AS 110 vial holder 96 low, start-up kit', p/n 191.0600).

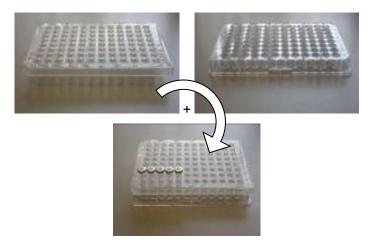


Figure 8: Left: transparent 96-positions plate, and right: 96-positions flat bottom PCR plate. The two plates can form a stabile holder for narrow fraction collector vials (lower picture, showing 5 inserted vials).

Configuration options

AS 110 standard

The following factory-installed options are available for the AS 110 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.
- Prep version: AS 110 suitable for large volume sampling. Because larger volumes must be injected for the Prep mode (see "Specifications Prep version" on page 52), AS 110 is fitted with a 2500 µL syringe and a 10000 µL sample loop.
- UHPLC version: up to 15,000 psi (~1000 bar) for the included 6port valve, and 10,000 psi (~690 bar) for the included 10-port UHPLC valve.

The following user-installable options are available:

•	Bio-compatible sample flow path and valve	Inert sample needle (Silco steel) and bio-compatible valve (PEEK)
•	Prep Kit	2.5 mL syringe, Prep valve, 10 mL sample loop, LSV needle and sample tray for 10 mL vials
•	Air needles	6 different types of air needles are available for the AS 110, each for a different type of well/vial plate. However, it is not just the type of well/vial plate that determines which air needle must be used. Refer to the section on <i>Air needles</i> for more information.
•	Valve unit	Special valve unit that can be replaced quickly and easily.

AS 110 micro

The following **factory-installed** option are available for the AS 110 micro 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. Temperature range inside the sampling compartment: 4 - 22°C.
- *ISS valve:* in the sampling compartment. This valve can either be used to connect extra solvents, or as a column selector.
- SSV valve: in the left panel of the autosampler, connected to the syringe wash position. Allows you to select multiple wash solvents.
- UHPLC version: up to 15,000 psi (~1000 bar) for the included 6port valve, and 10,000 psi (~690 bar) for the included 10-port UHPLC valve.

The following user-installable options are available:

٠	Bio-compatible sample	Inert sample needle (coated steel) and
	flow path and valve	bio-compatible valve (PEEK/ceramic)

- Air needles6 different types of air needles are available for the AS 110 micro, each for a
different type of well/vial plate. However,
it is not just the type of well/vial plate that
determines which air needle must be
used. Refer to the section on Air needles
for more information.
- Valve unit Special valve unit that enables easy replacement of valves.

Injections

The autosampler is equipped with a low pressure flow path, a compressor a sampling valve and three standard injection modes and an option for user defined programming:

- Full loop injections: for maximum precision
- Partial loop fill injections: for maximum flexibility
- µL Pickup injections: for zero sample loss
- User Defined Program: for full flexibility

These injection modes accommodate use of a wide variety of applications and are explained in full detail in this chapter.

Pressure-Assisted Sample Aspiration

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.

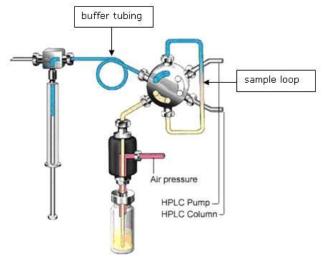


Figure 9: PASA injection concept.

Syringe and buffer tubing

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.

Three sizes of syringes are available <u>for the AS 110 standard</u>: 250 (FW 1.26 >), 500 and 2500 μ L (prep version). Using the 500 μ L syringe combined with 1000 μ L buffer tubing and 100 μ L sample loop, the following inj. volume range is available for the various injection modes:

- Full loop : 100 µL
- Partial loopfill : 0 50 µL
- μL pick-up : 0 27 μL

<u>For the AS 110 micro</u> three sizes of syringes are available: $25 \ \mu$ L, $50 \ \mu$ L and $100 \ \mu$ L. The $25 \ \mu$ L syringe is the standard syringe; combined with the 50 μ L buffer and the 20 μ L sample loop the following inj. volume range is available for the various injection modes:

- Full loop : 20 µL
- Partial loopfill : 0 20 µL
- μL pick-up : 0 6.4 μL

For other combinations of loop, syringe and tubing, the maximum inj. volumes are calculated with the following formulas:

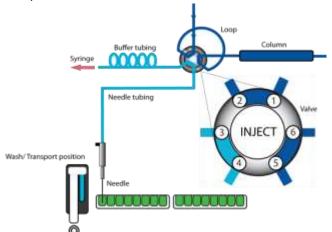
- Full loop : injection volume = loop volume
- Partial loopfill : max. inj. volume = $\frac{1}{2}$ x of loop volume
- µL Pick up : max. inj. volume = (loop volume 3 x needle volume)/2

Injection principles: Full loop injection

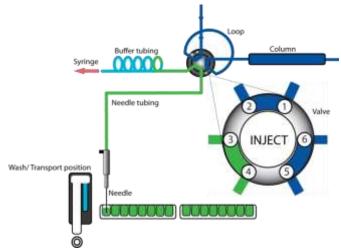
<u>*Full loop*</u> gives maximum possible reproducibility < 0.3%, but not maximum accuracy, since the loop volume is specified with an accuracy of \pm 10%.

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

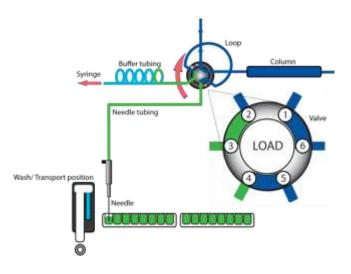
 The initial situation: the injection valve is in INJECT position. The sample needle with air needle has entered the well or vial. Headspace pressure, applied through the air needle, ensures that no air or vapour bubbles are formed during sample aspiration.



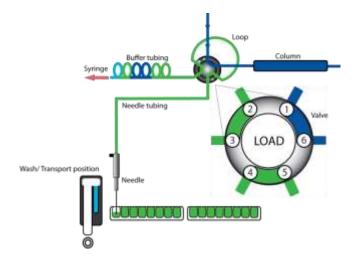
2. The syringe dispenser aspirates the "flush volume" from the sample well/vial to fill the sample line with sample and remove wash solvent.



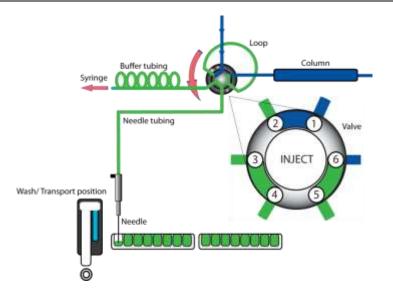
3. The injection valve is switched to LOAD position, placing a distinct sample plug at the inlet of the sample loop.



- 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 \leq 100 µL
 - 2 x loop volume for loops 100 μL 500 μL
 - 1.5 x loop volume for loop $> 500 \; \mu L$



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



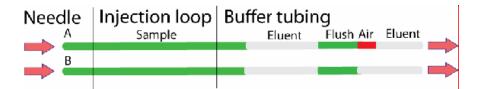
A wash routine is performed after each injection.

Air segment with Full loop injections

An air segment of $5 \mu L$ (0.5 μL for micro version) can be used to minimize mixing of sample during aspiration. This air segment is at the front of the flush volume and will not be injected.

With a standard 15 μ L needle, the flush volumes must be a minimum of 30 μ L + air segment (5 μ L) and 30 μ L for injections without air segment. With a 2.4 μ L micro needle, the flush volumes must be a minimum of 4.8 μ L + air segment (0.5 μ L) and 4.8 μ L 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.

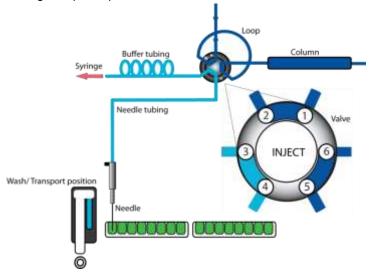


Injection principles: Partial loopfill injection

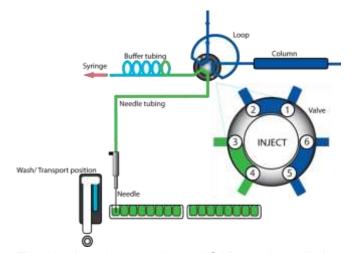
<u>Partial loopfill</u> gives maximum flexibility, maximum accuracy plus reproducibility better than 0.5% RSD for injection volumes > 10 µL for the AS 110 standard. For the AS 110 micro the reproducibility is better 1.0% RSD for injection volumes > > 0.5 µL. Minimum sample required (Flush volume) = 30 µL (AS 110 std) or 4.8 µL (AS 110 micro). These are the recommended minimum flush volume, smaller flush volumes can be programmed, but will result in decreasing performance.

The switching sequence for a partial loopfill injection is:

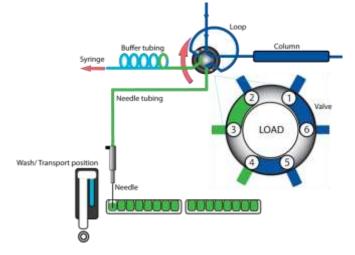
1. The initial situation: the injection valve is in the INJECT position. The sample needle with air needle has entered the vial/well. Headspace pressure, applied through the outer air needle, ensures that no air or vapour bubbles are formed during sample aspiration.



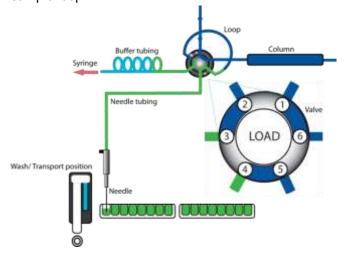
2. The syringe dispenser aspirates the "flush volume" from the sample vial to fill the sample line with sample and remove wash solvent.



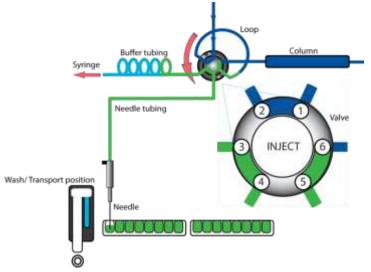
3. The injection valve switches to LOAD, placing a distinct sample plug at the beginning of the sample loop.



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 HPLC mobile phase flow path: sample is transported to the column. The analysis starts.

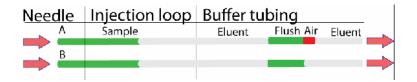


If an injection from the same vial and no wash routine is programmed, the next injection sequence will start with a flush of 50% of the programmed flush volume. Otherwise, it will start with a flush of the programmed flush volume. If the withdrawal of sample for the next injection exceeds the total volume 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 of $5 \mu L$ (0.5 μL for micro version) can be used to minimize mixing of sample during aspiration. This air segment is at the front of the flush volume and will not be injected.

With a standard 15 μ L needle, the flush volumes must be a minimum of 30 μ L + air segment (5 μ L) and 30 μ L for injections without air segment. With a 2.4 μ L micro needle, the flush volumes must be a minimum of 4.8 μ L + air segment (0.5 μ L) and 4.8 μ L 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.

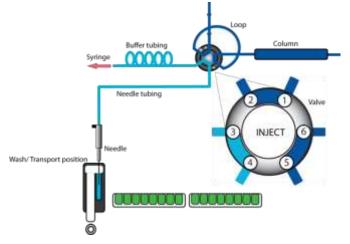


Injection principles: µL Pickup injection

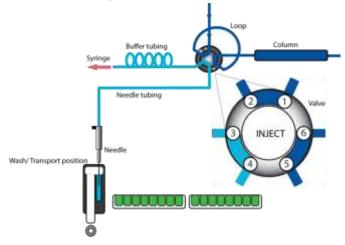
<u>*Pick-up*</u> offers no sample loss, maximum accuracy (same as partial loopfill), but slightly lower reproducibility: AS 110 standard: RSD better than 1% for injection volumes > 10 μ L AS 110 micro: RSD better than 2.5% for injection volumes > 0.5 μ L

The switching sequence for µL pickup injections is:

1. In the initial situation, the injection valve is in INJECT position. The sample needle has entered the transport position.

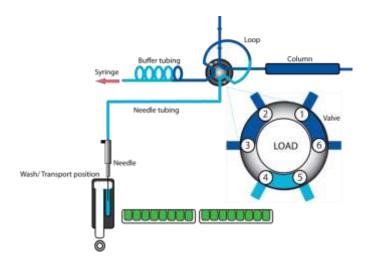


2. The transport reservoir is filled with wash solvent the programmed amount of times the syringe volume (after a wash or after emptying of buffer tubing). The injector valve remains in INJECT position during fill transport. Please note that the transport solvent needs to be compatible with eluent.

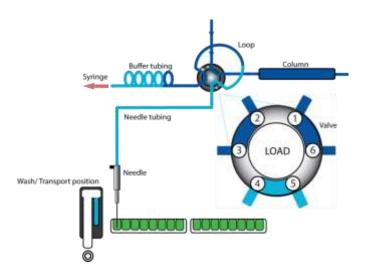


Introduction

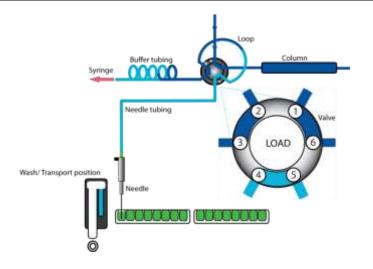
3. For the first injection, the syringe dispenser aspirates a transport plug from the transport position to fill the sample line with transport liquid and remove wash solvents.



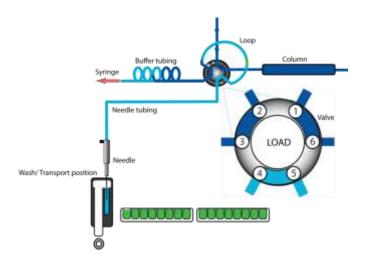
4. The needle moves from the transport position to the sample vial. The injection valve switches to LOAD position.



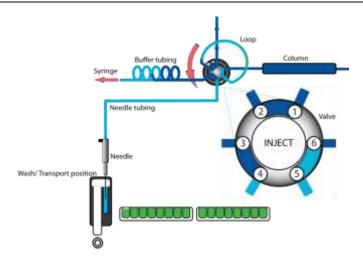
5. The programmed injection volume is aspirated from the sample vial.



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 HPLC mobile phase flow path: sample is transported to the column. The analysis timer starts.

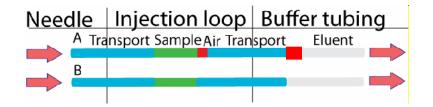


The sequence is repeated for each injection.

Air segment with µL Pickup injections

If an air segment (5 μ L or 0.5 μ L for an AS 110 standard and AS 110 micro respectively) 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 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.



Syringe speed scale factor

The standard injection procedures use a programmable syringe speed. The tables below show the flow rates for each code.

AS 110 micro

	LOAD (µL/min)			UNLOAD (µL/min)		
Scale factor	Low	Normal	High	Low	Normal	High
0.1	3	6	9	14	27	34
0.2	6	13	19	27	53	69
0.3	9	19	28	41	80	102
0.4	13	25	37	55	107	137
0.5	16	31	47	69	133	171
0.6	19	37	56	81	160	209
0.7	22	44	65	96	185	240
0.8	25	49	75	109	218	282
0.9	28	56	84	123	240	320
1.0	31	62	94	137	267	343

For the 25 µL syringe (factory installed) the following scale factors apply:

For the 50 μL syringe the following scale factors apply:

	LOAD (µL/min)			UNLOAD (µL/min)		
Scale factor	Low	Normal	High	Low	Normal	High
0.1	6	13	19	27	53	68
0.2	13	25	38	55	106	137
0.3	19	38	56	82	160	204
0.4	25	50	74	109	213	274
0.5	31	62	93	137	267	343
0.6	38	74	112	163	320	417
0.7	44	87	130	192	369	480
0.8	50	99	150	218	436	565
0.9	56	112	168	246	480	640
1.0	62	125	188	274	533	686

	LOAD (uL/min)		UNLOAD (µL/min)		
Scale factor	Low	Normal	High	Low	Normal	High
0.1	13	25	38	55	106	136
0.2	25	50	75	109	211	275
0.3	38	75	112	164	320	409
0.4	50	100	149	218	427	549
0.5	63	125	187	274	533	686
0.6	75	149	223	325	640	835
0.7	87	175	259	384	738	960
0.8	100	198	300	436	873	1129
0.9	112	223	337	492	960	1280
1.0	125	249	377	549	1067	1371

For the 100 μL syringe the following scale factors apply:

AS 110 standard

For the 250 μL syringe (FW > 1.26) the following scale factors apply:

	LOAD (µL/min)			UNLOAD (µL/min)		
Scale factor	Low	Normal	High	Low	Normal	High
0.1	32	63	95	137	265	340
0.2	63	126	188	273	528	686
0.3	95	188	281	410	800	1022
0.4	126	250	372	545	1067	1372
0.5	157	312	467	686	1333	1714
0.6	188	372	558	813	1600	2087
0.7	218	437	648	960	1846	2400
0.8	250	495	750	1091	2182	2823
0.9	281	558	842	1231	2400	3200
1.0	312	623	942	1372	2667	3428

	LOAD (uL/min)		UNLOAD (µL/min)		
Scale factor		Normal	High	Low	Normal	
0.1	63	125	189	274	530	680
0.2	125	251	375	545	1055	1371
0.3	189	375	561	820	1600	2043
0.4	251	500	744	1090	2133	2743
0.5	314	624	933	1371	2666	3428
0.6	375	744	1116	1626	3200	4174
0.7	436	873	1296	1920	3691	4800
0.8	500	989	1500	2181	4363	5646
0.9	561	1116	1684	2461	4800	6400
1.0	624	1246	1883	2743	5333	6856

For the 500 μ L syringe the following scale factors apply:

Mix & dilute

A Mix & Dilute routine can be created for the AS 110. This routine allows you to process the sample before injection. You can program three different types of actions:

 Add the indicated volume from the Sample/Reagent A/Reagent B/Wash position and dispense it to the Sample/Destination position.



To prevent cross-contamination, the AS 110 will aspirate an additional volume of 25% of the programmed volume to flush the tubing and needle. The aspirate and dispense speed depends on the selected syringe and the programmed syringe speed.

- Example: ADD 20 uL from Reagent A to Destination will result in the following actions:
- Aspirate an air segment of 0.5 µL to separate the wash solvent in the buffer tubing from Reagent A.
- Aspirate 5 µL Reagent A to flush the tubing and needle.
- 4. Empty the syringe to the syringe-waste position.
- Aspirate 20 µL Reagent A and dispense it to the destination vial.
- 6. Rinse buffer tubing and needle with wash solvent.
- **Mix** (aspirate and dispense) a number of the times the programmed volume from the destination vial. If no destination vial is available, the mix is performed in the sample vial.

Example: Mix 3 times with 25 µL will result in the following actions:

- 1. Aspirate an air segment of 0.5 µL to separate the wash solvent in the buffer tubing from the solvent to be mixed.
- 2. Empty the syringe to the syringe-waste position.
- Aspirate 25 µL solvent and dispense it back into the vial/well.
- 4. Repeat step number 3 twice.
- 5. Rinse buffer tubing and needle with wash solvent.

The mix is performed from the destination position when the previous ADD action is TO DESTINATION. When the previous ADD action is TO SAMPLE, the mix is performed from the sample position.

• Wait the programmed period of time before continuing with the next step (reaction time). H:MM:SS, maximum of 9 hours, 59 minutes and 59 seconds.

A maximum of 15 steps can be created for the Mix & Dilute routine.

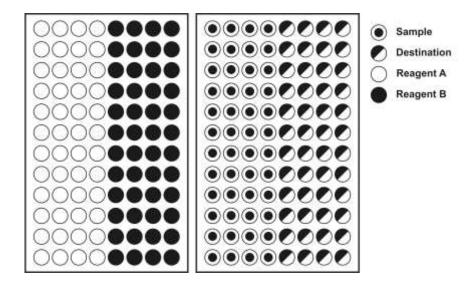
Sample positions Mix & Dilute



If a mix is programmed, the reagent solvents, destination and sample positions in the trays are as follows:

- Reagent A: Left plate
- Reagent B: Left plate
- Samples: Right plate
- Destination: Right plate

If you chose to process plates in rows, the following positions are available for sample, destination, reagent A and reagent B:



For example, you can program the following:

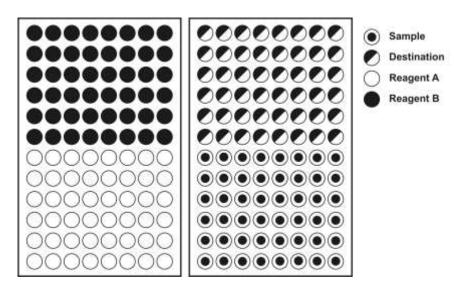
- two 96-low plates, processed in rows
- first line: first sample position 2A1, last sample position 2A1, destination position 2E1, Reagent A 1A1 and Reagent B 1E1
- second line: first sample position 2A2, last sample position 2A2, destination position 2E2, Reagent A 1A2 and Reagent B 1E2

 third line: first sample position 2A3, last sample position 2A3, destination position 2E3, Reagent A 1A3, Reagent B 1E3.

However, if you were to use a 12-vial tray for the Reagent, you could use reagent from the same vial a number of times. For example:

- first line: first sample position 2A1, last sample position 2A1, destination position 2E1, Reagent A 1A1 and Reagent B 1E1
- second line: first sample position 2A2, last sample position 2A2, destination position 2E2, Reagent A 1A1 and Reagent B 1E1
- third line: first sample position 2A3, last sample position 2A3, destination position 2E3, Reagent A 1A1, Reagent B 1E1, etc.

If you chose to process plates in columns, the following positions are available for sample, destination, reagent A and reagent B:



For example, you can now program the following:

- two 96-low plates, processed in columns
- first line: first sample position 2A1, last sample position 2A1, destination position 2A7, Reagent A 1A1 and Reagent B 1A7
- second line: first sample position 2B1, last sample position 2B1, destination position 2B7, Reagent A 1B1 and Reagent B 1B7
- third line: first sample position 2C1, last sample position 2C1, destination position 2C7, Reagent A 1C1, Reagent B 1C7, etc.

The mix method can be executed before the injection method.

- The injection is performed from the destination position when a mix method is programmed and TO DESTINATION is the last step in the mix method.
- The injection is performed from the sample position when a mix method is programmed and TO SAMPLE is the last step in the mix method.

The mix method can also be executed without an injection method.

User Program

The user program mode is available for the AS 110 micro, and for the AS110 standard with FW 1.26 or higher in combination with Clarity software version 4 or higher.

The User Program offers the possibility to program all possible actions required for a sample handling sequence in separate steps.

The following User Program steps are available:

ASPIRATE	a programmed volume from sample well, ambient air, destination vial, wash, or one of the reagent vials into the buffer tubing. Speed and height of syringe can be entered (refer to table below). The maximum volume that can be aspirated is the total volume of the syringe.
DISPENSE	a programmed volume from the buffer tubing into the sample well, waste , destination vial, wash or one of the reagent vials. Speed and height of syringe can be entered (refer to table below). It is not possible to dispense a larger volume than the total volume aspirated in previous actions.
SYRINGE VALVE	to program the position of the syringe to one of its three tubes: NEEDLE: connection to sample needle WASH: connection to wash solvent bottle WASTE: connection to syringe waste tubing.
SYRINGE	to control the action of the syringe: LOAD: the syringe with the programmed volume UNLOAD: the syringe with the programmed volume HOME: the volume previously aspirated will be dispensed to the last programmed position, and the syringe will be initialized again.
NEEDLE WASH	to execute a needle wash; the content of the buffer tubing is not rinsed to waste before the start of the wash. The programmed volume of wash solvent is used to wash the needle at the wash position. The wash position may be contaminated with the contents of the buffer tubing, which may generate cross- contamination. To prevent contamination of the wash



position, program a dispense to waste action before programming a wash action.

VALVE to program positions of high pressure valves (ISS or injector valve). The injector valve has two positions: <INJECT> and <LOAD>. The ISS optional valve has positions 6-1 and 1-2:

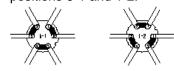
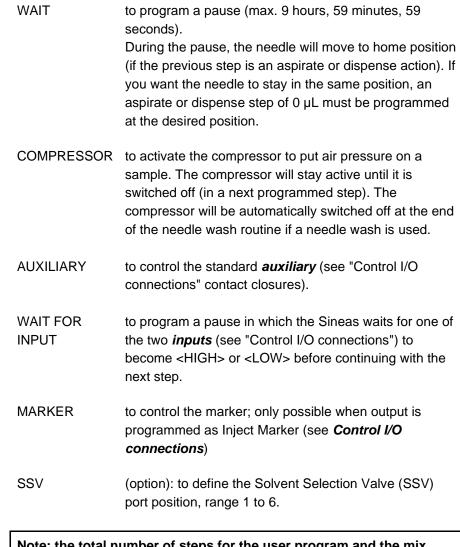


Figure: ISS valve positions







Note: the total number of steps for the user program and the mix method cannot exceed 240.

SPEED	SYRINGE		
	25 µL	50 µL	100 µL
1	3 µL/min	6 µL/min	13 µL/min
2	13 µL/min	25 µL/min	50 µL/min
3 (LOW)	31 µL/min	63 µL/min	126 µL/min
4 (NORMAL)	62 µL/min	125 µL/min	250 µL/min
5 (HIGH)	94 µL/min	188 µL/min	377 µL/min
6	192 µL/min	384 µL/min	768 µL/min
7	267 µL/min	533µL/min	1067 µL/min
8	343 µL/min	686 µL/min	1371 µL/min
9	436 µL/min	873 µL/min	1745 µL/min



During the dispense action the pressure in the buffer tubing will increase. To prevent damage of the syringe valve, the flow should not exceed the value of 250 μ L/min for water. (Maximum speed 6 for a 25 μ L syringe, speed 5 for a 50 μ L syringe and speed 4 for a 100 μ L syringe. If more viscous liquids are used the speeds should be reduced.

Example

The following example is valid for the AS 110 micro, configured with the listed items below:

- 1.5 µL sample loop
- 2.4 µL needle
- 25 µL syringe

The example of a user defined program (Table 2) will perform an in-needle derivatisation procedure (e.g. for GABA-Glu analysis), followed by an injection according to the schematic principle depicted in Figure 10.

As	pirate air plug for	insulation from	n wash solution	Schematics of fluid
1	Aspirate	Air	2 uL	Q
IA	spirate sample an	d reagent		
2	Aspirate	Reagent A	0.5 uL	
3	Aspirate	Sample	9 uL	D
III N	Nove sample to the	e buffer tubing		
4	Aspirate	Air	12 uL	
IVA	lixing			
5	Dispense	Waste	10 uL	
6	Aspirate	Air	10 uL	
7	Dispense	Waste	10 uL	
Vir	ject and wash			↔
8	Injector valve	LOAD		Sample
9	Dispense	Waste	7 uL	loop
10	Injector valve	INJECT		,000
11	Needle wash		250 uL	· •

Figure 10: Principle of automated 'in needle' derivatization procedure.

Table 2: User defined program for in-needle derivatisation procedure

Step	Program	Explanation
1	Syr.Speed/Height Speed: 4, Height: 3.5 mm	
2	Aspirate 2.00 µL Air, Speed: 4, Height: 3.5 mm	I Aspirate air plug for insulation
3	Wait 0.05 [min]	
4	Syr.Speed/Height Speed: 1, Height: 5.0 mm	
5	Aspirate 0.50 µL Reagent A, Speed: 1, Height: 5.0 mm	II Aspirate reagent
6	Wait 0.05 [min]	
7	Syr.Speed/Height Speed: 2, Height: 3.5 mm	
8	Aspirate 9.00 µL Sample, Speed: 2, Height: 3.5 mm	II Aspirate sample
9	Wait 0.05 [min]	
10	Syr.Speed/Height Speed: 2, Height: 3.5 mm	
11	Aspirate 2.00 µL Air, Speed: 2, Height: 3.5 mm	
12	Wait 0.05 [min]	
13	Syr.Speed/Height Speed: 4, Height: 6.0 mm	
14	Dispense 0.00 µL to Waste, Speed: 4, Height: 6.0 mm	
15	Syr.Speed/Height Speed: 4, Height: 6.0 mm	
16	Aspirate 10.00 µL Air, Speed: 4, Height: 6.0 mm	III Move sample to the buffer tubing
17	Syr.Speed/Height Speed: 4, Height: 6.0 mm	
18	Dispense 10.00 µL to Waste, Speed: 4, Height: 6.0 mm	IV Mixing steps
19	Syr.Speed/Height Speed: 4, Height: 6.0 mm	
20	Aspirate 10.00 µL Air, Speed: 4, Height: 6.0 mm	
21	Syr.Speed/Height Speed: 4, Height: 6.0 mm	
22	Dispense 10.00 µL to Waste, Speed: 4, Height: 6.0 mm	
23	Wait 0.05 [min]	
24	Valve Injector, Position: Load	
25	Syr.Speed/Height Speed: 2, Height: 6.0 mm	
26	Dispense 7.00 µL to Waste, Speed: 2, Height: 6.0 mm	Position the sample in the sample loop
27	Wait 0.10 [min]	
28	Valve Injector, Position: Inject	V Inject
29	Markers Analog Inject	
30	Needle wash 300.00 µL	V Wash

CHAPTER 2

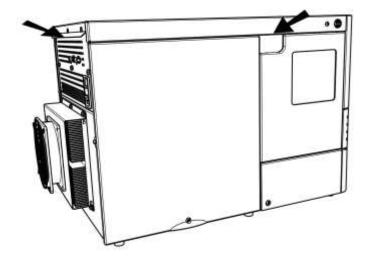
Installation

Unpacking

Inspect the *transport box* for possible damage as it arrives. Immediately inform the transport company in case of damage, otherwise she may not accept any responsibility. Keep the transport box as it is designed for optimum protection during transport and it may be needed again. Carefully unpack the autosampler and inspect it for completeness and for possible damage. Contact your supplier in case of damage or if not all marked items on the checklist are included.Prior to shipment, your autosampler has been thoroughly inspected and tested to meet the highest possible demands.

Execute the following steps for initial installation of the AS 110 autosampler:

1. Lift the AS 110 from its packaging using both hands at the marked position.



2. With both hands under the instrument, lift the AS 110 to its operating location. Keep the instrument upright.



Position the Alias so that it is possible to access the power plug. Always remove the power cable from the instrument before opening the cover.

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 AS 110, this may also influence the cooling capabilities.

Objects can be placed on any side of the AS 110; however make sure that the distance of any objects at the back side of the AS 110 is sufficient to assure optimal ventilation (Peltier cooling):

 \bullet 5 cm from the AS 110, if objects are placed at only one side of the AS 110

• 10 cm from the AS 110, if objects are placed on more than one side of the AS 110

Do not place the AS 110 in an area subject to excessive dust or shocks. Use the AS 110 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.

- 3. Leave the AS 110 to adopt ambient temperature for at least one hour.
- 4. Install AS 110 Service Manager (see "ASM software" on page 45) on your PC.
- 5. Check that fuses and voltage range on the rear side of the instrument match that for the power outlet to be used.



Understanding power surges Power surges, line spikes, and transient energy sources can impede instrument operation. Ensure that the instrument's electrical supply is adequately protected from these conditions and properly grounded.

- Connect the AS 110 to the PC COM-port with the cable provided with the AS 110. Or to a LAN port on a LAN switch using the LAN cable provided with the instrument (L versions of the AS110). A LAN switch is delivered when a complete ALEXYS system is purchased.
- 7. Connect the power cable between the AS 110 and the power outlet.



Power supply and protective earth. The system must be connected to a suitable mains power supply with a correctly installed protective earth conductor. Never use the system without a properly connected protective earth conductor.

- 8. Switch on the AS 110.
- 9. On your PC, open AS 110 Service Manager and enter the required settings.
- 10. Connect the drain tubing to the waste outlet.
- 11. Fill the wash solvent bottle inside the sampling compartment of the AS 110 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.
- 12. 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.

- 13. Check if air bubbles are trapped in the syringe; remove them by gently tapping the syringe.
- 14. 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.

ASM software

A software package is supplied with the AS 110 autosampler: AS 110 service manager (ASM). AS 110 Service Manager allows you to upload AS 110 firmware. It offers the following functionality:

- You can define a port through which you want to do the update.
- You can access the Communication Settings, Direct control, Service and Adjustment windows for AS 110.
- You can access ASM help and About.

Execute the following steps to install ASM:

- Check the Antec website for the latest version and store it to a convenient location, or insert the Antec USB drive into a free USB port of your PC.
- 2. If the autorun feature is active, the installation wizard will appear. If autorun is not active, use the browser to go to the USB stick and double click install.exe to start the installation wizard.
- 3. Answer all questions that pop up in the wizard; click Next to go to the next step in the installation procedure.
- 4. Click Finish to end the installation procedure.
- 5. The software is now installed. Refer to online help of ASM for more information.

Upload notification. If the upload notification is displayed, the ASM has discovered a problem in PC settings that prevents correct uploading of the new files.

This problem may be solved by adapting the size of the serial ports FIFO.

Execute the following steps to adapt settings:

- 1. Open the Windows Device Manager (Configuration settings/System settings/Communication port)
- 2. In the COM port properties, select the Port settings tab.
- 3. Click Advanced.
- Use the sliders to change the size of the receive buffer. The default values for Receive buffer high (14) and Transmit buffer high (16) should be sufficient. However, decrease the values to 8 if buffer overflow errors appear.
- 5. Click OK and close the Windows Device Manager.
- 6. Restart your PC.
- 7. Open ASM and try to upload again.
- 8. Contact service if there still are problems during uploading.

Setting up a LAN connection

Follow the instructions in this paragraph when the instrument is used with PC control over LAN using the ASM or Clarity Chromatography Software. To be able to communicate over LAN a computer is required with a free (PCI, PCI Express or PCI-X) LAN port + a LAN Gigabit switch.

If the instrument is delivered as a part of a complete ALEXYS (U)HPLC-ECD system an additional LAN connectivity kit (pn 250.0180) which consist of a set of network cables and LAN gigabit switch is available. In that case please refer to the detailed installation instructions in document 250.7018 LAN connection kit installation guide.



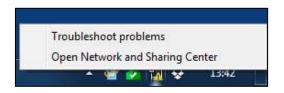
To insure stable and error-free communication it is advised to use the manufacturer-supplied LAN connectivity kit to connect the AS110 to LAN. The LAN connectivity kit (pn 250.0180) can be purchased separately. Do <u>not</u> connect the AS110 over a company Local Area Network. If needed a second network adapter with a different (unique) IP address range can be applied.

The AS110 (L version) has a fixed IP address: 192.168.5.2, with subnet mask: 255.255.255.0. Gateway and DNS are not filled in.

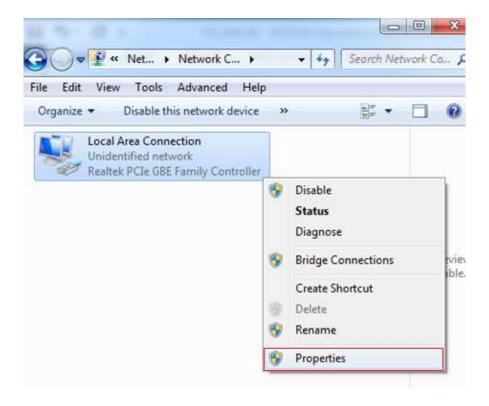
Configure the IP address of the PC LAN network card by executing the following steps (in this example the Windows 7 OS is used, in Windows 8 the example screens may look different):

1. Right click on the Network icon in the bottom right of the Windows taskbar and open the menu 'Open Network and Sharing Center'.

Alternative: open Windows start menu, open control panel and goto 'Open Network and Sharing Center'):



- 2. Open the menu 'Change adapter settings' on the left panel of the 'Open Network and Sharing Center' window.
- 3. Right click on the Local Area Connection icon of the LAN card in your PC and click on properties to open the Network card setting.



Chapter 2

letworking	Internet Protocol Version 4 (TCP/I	Pv4) Properties
Connect using:	General	
Realtek PCIe GBE Family Controller Configure		automatically if your network supports sed to ask your network administrator
This connection uses the following items:	Obtain an IP address autom	atically
Client for Microsoft Networks	• Use the following IP address	
QoS Packet Scheduler	IP address:	192.168.5.10
File and Printer Sharing for Microsoft Networks Anternet Protocol Version 6 (TCP/IPv6)	Subnet mask:	255.255.255.0
✓ Internet Protocol Version 4 (TCP/IPv4) ✓ ▲ Link-Layer Topology Discovery Mapper I/O Driver	Default gateway:	• • •
🗹 🛶 Link-Layer Topology Discovery Responder	Obtain DNS server address	
Install Uninstall Properties	Output Description of the server of the s	r addresses:
Description	Preferred DNS server:	
Transmission Control Protocol/Internet Protocol. The default wide area network protocol that provides communication	Alternate DNS server:	
across diverse interconnected networks.	Vaļīdate settings upon exit	Ad <u>v</u> anced
OK Cancel		OK Cancel

- 4. Open the menu 'Internet Protocol Version 4 (TCP/IPv4)' menu (double click).
- Configure the network IP address and subnet mask as depicted in the screen dump below (IP 192.168.5.10, Subnet mask: 255.255.255.0). Gateway and DNS fields are not filled.
- Close the menu(s) by clicking the 'OK' buttons. The network IP address of the LAN network card is now set up for communication with the AS110 autosampler.
- Connect the AS110 via the supplied LAN cable to a LAN Gigabit switch which is connected to the configured LAN port on the control PC.
- 8. Switch on the AS110 to establish a LAN connection.
- 9. Start-up the ASM software, or DataApex Clarity. In the figure on the next page a screen dump is shown of the Communications window of the ASM. As can be seen the autosampler is available under the Ethernet tab with IP address 192.168.5.2. Select TCP communication to make a remote connection

Communication			×
Com port Ethernet USB Protocol TCP/IP UDP/IP			
Mac address	Ip address	Device name	
🏫 00-40-9D-54-9B-83	192.168.5.2		
Refresh			
Select Cancel	Help		

Figure 11: Screen dump of Ethernet tab under the communications window of the ASM PC software.

Below the AS110 (Alias) configuration window is shown in DataApex Clarity. When selecting the AS110 control driver in Clarity choose the 'Alias driver, by Spark Holland including user program'.

Alias Setup		×
Communication	⊖rs232 ⊚lan	OUSB
Serial Port	COM1	\sim
IP Address	192 . 168 . 5 . 2	
USB Port	No port available	\sim
ID	61	AutoDetect
	Status: Unknown	
Sampler Name	Sampler 1	
	Sequence Mode	
	Check Door	
	Enable Alphanumeric Vial Names	
	Digital Input Names	Change
OK	Cancel	Help

Figure 12: Screen dump of the configuration window in DataApex Clarity.

AS 110 flow path

Refer to Table 1 for the details of the factory installed items in the flow path for an AS 110 autosampler with a 6-port valve (Figure 13), and a 10-port valve (Figure 14).

Notice that the waste line is not connected to the left side port of the syringe valve but on the backside port, which is not visible and accessible without loosening/removing the syringe valve. *Note*: with the AS 110 standard (FW > 1.26) it is possible to use the left side port of the syringe valve to connect a second wash bottle(supported in the AS 110 Clarity driver developed by DataApex/Spark).

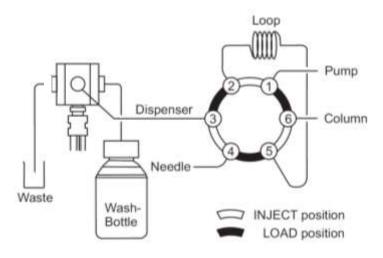


Figure 13: AS 110 fluid connections to a 6-port valve.

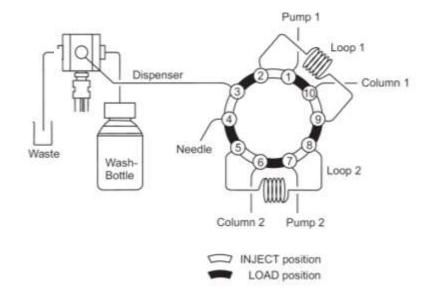


Figure 14: AS 110 fluid connections to a 10-port valve of an AS110 standard <u>HPLC</u> 10-PV autosampler. Part number 191.0041(L).

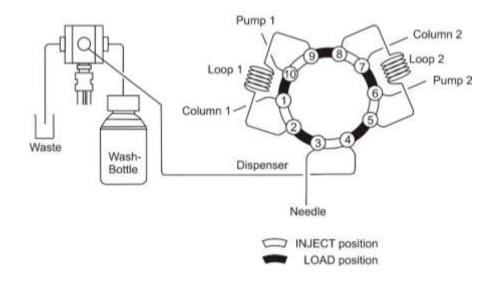


Figure 15: AS 110 fluid connections to a 10-port valve of an AS110 standard <u>UHPLC</u> 10-PV autosampler. Part number 191.0041U(L).Note that the port orientation and configuration differs from the HPLC version.

An 10-port autosampler can also be changed into a one channel configuration using a single channel conversion kit. For example for the AS110 10-PV UHPLC the following kit is available: 10-p UHPLC conversion kit, single channel (pn. 250.0034A):

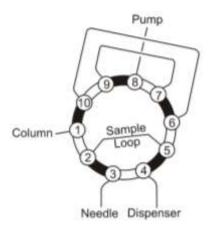


Figure 16: Fluid connections to a 10-port valve of an AS110 10-PV **<u>UHPLC</u>** autosampler, configured as single channel autosampler with one sample loop.

nstallation

When making connections to the autosampler sampling valve, make sure that the tubing is correctly connected as depicted in the figures on the previous pages.

AS 110 tubing details

If you need to install new tubing:

- insert tube ends always flush with ferrule ends
- do not overtighten nuts, it 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.

The AS 110 standard is fitted with the following tubing:

Tubing	Materials/Dimensions
Standard sample needle and tubing (label 15 $\mu L)$	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
Buffer tubing from high-pressure valve to syringe valve (label 1000 μL)	ETFE (Tefzel): 1275 mm x 1/16" OD x 1.0 mm ID
Syringe valve to wash solvent bottle	PTFE: 400 mm x 1/8" OD x 1.6 mm ID
Syringe valve to waste	PTFE: 400 mm x 1/8" OD x 1.6 mm ID

The AS 110 micro is fitted with the following tubing:

Tubing	Materials/Dimensions
Standard sample needle and tubing	SS: 136 mm x 0.8 mm OD x 0.1 mm ID
(label 2.4 µL)	PEEKsil: 165 mm x 1/32" OD x 0.1 mm ID
Buffer tubing from high-pressure valve to syringe valve (label 50 μL)	PEEK: 260 mm x 1/16" OD x 0.5 mm ID
Syringe valve to wash solvent bottle	PTFE: 400 mm x 1/16" OD x 0.75 mm ID
Syringe valve to waste	PTFE: 400 mm x 1/8" OD x 1.6 mm ID

Tubing	Materials/Dimensions
Standard sample needle and tubing	SS: 136 mm x 0.8 mm OD x 0.1 mm ID
(label 2.4 µL)	PEEKsil: 165 mm x 1/32" OD x 0.1 mm ID
Buffer tubing from high-pressure valve to syringe valve (label 50 μL)	PEEK: 260 mm x 1/32" OD x 0.5 mm ID
Syringe valve to wash solvent bottle	PTFE: 400 mm x 1/16" OD x 0.75 mm ID
Syringe valve to waste	PTFE: 400 mm x 1/8" OD x 1.6 mm ID

The AS 110 micro UHPLC is fitted with the following tubing:

The AS 110 UHPLC is fitted with the following tubing:

Tubing	Materials/Dimensions
Standard sample needle and tubing (label 15 $\mu L)$	SS: 136 mm x 0.8 mm OD x 0.25 mm ID PEEK: 165 mm x 1/32" OD x 0.25 mm ID
Buffer tubing from high-pressure valve to syringe valve (label 200 μL)	PEEK: 1020 mm x 1/32" OD x 0.5 mm ID
Syringe valve to wash solvent bottle	PTFE: 400 mm x 1/16" OD x 0.75 mm ID
Syringe valve to waste	PTFE: 400 mm x 1/8" OD x 1.6 mm ID

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:

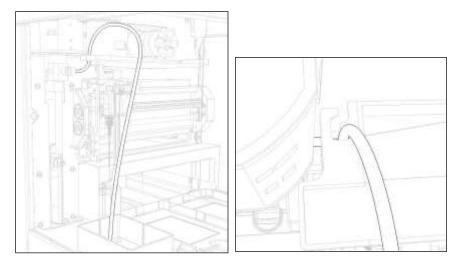


Figure 17: Tubing guide (I) and top view of tubing guide (r).

Waste tubing

Make the following connections for disposal of waste liquids:

- General waste: connect the drain tubing (in the ship kit of the AS 110) to the right-hand drain hose connector (see Figure 2, 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 2, 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 ship kit of the AS 110) 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 AS 110. You are recommended to use electrochemically clean water (R<18MOhm-cm), or a mixture of such water and iso-propanol (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 in this solution; 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. Open Direct Control in AS 110 Service Manager.
- 3. In the Syringe group 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 contents is dispensed to syringe waste.

- 5. Repeat steps 3 and 4 until the wash solvent tubing and the syringe are completely filled.
- When wash solvent tubing and syringe are completely filled, click Start in the Initial wash group box to perform a standard wash routine. All tubing connected to the syringe valve will be rinsed with wash solvent.
- 7. Click Close to leave the Direct control screen. The AS 110 is initialized.

8. In case an air bubble is trapped at the tip of the syringe or in the tubing, perform these steps with the wash solvent tubing end in 100% methanol. When the bubble is removed, repeat again with the final original wash solvent.

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/wells 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:
 - o for standard (low) well plates: sealing tape
 - for deep well plates: pierce-able capmats (Pre-slit or silicon) or sealing tape
 - 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/wells, 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.

CHAPTER 3

Getting started

This chapter describes how to get started with the AS 110 using the DataApex Clarity software. The following will be explained:

- Menus of the AS 110
- Configuration in Clarity
- AS 110 method menu
- executing a series.

Menus of the AS 110

Important method/service menus in the Clarity AS 110 control module are:

- Mode, Time and Temp.: here the autosampler analysis time, injection mode, flush volume, wash volume, wash type and tray cooling temperature are defined.
- Input and Outputs: In this menu the functionality of the output (relay, analog inject marker and alarm) and inputs (next injection, Freeze, Stop) can be defined. The IO can be controlled by means of a programmable timed event table.
- Mix method: programming of simple mix & dilute methods.
- User program: advanced user-defined injection programming interface in which fully customized injection methods can be created. Dispense, aspirate, valve switching, IO actions can be programmed step-by-step in an event table.
- System settings: in this tab the AS 110 flow path (loop volume, needle volume, syringe volume etc) and injection parameters (syringe speed, headspace pressure, etc.) are defined for the standard AS 110 injection methods: full-loop, partial loopfill, uL-pickup.
- **Tray**: allows you to define which trays or well plates are present in the autosampler, tray/well plate processing direction, first destination vial and reagent vial (A-D) positions.
- **Device monitor:** under the AS 110 micro autosampler tab in the device monitor window important functionality is available for service and maintenance on the autosampler flow path.



Note that the AS 110 standard and AS 110 micro use a different control module in Clarity.

Device monitor

The device monitor is an important menu for the maintenance and service of the AS 110 by the end-user. Some important actions can be performed in this menu such as (for the AS110 micro these windows look different):

- The injection valve can be checked/switched using "Load/Inject"
- The syringe can be moved to its exchange position to be able to remove it for service or replacement (Syringe operation 1).
- Initial wash, to remove air bubbles from the flow path
- Set tray cooling temperature
- Tray position (Home/Front)
- Needle position (Home/End/Exchange)

🥸 Instrument 1 - Device Monitor	– 🗆 X
File Control View Window Help 📐 👖 😤 着	i 🗉 🖻 🕸 💿 🏹 😭 🕑 🧿
Alias Sampler 1 (SN 100301)	Ready, 27 °C 🛛 🔘
Initial Wash - Start Reset error	AS Status
Switch Tray Cooling Off	Service
Tray Move - Front Tray Move - Home	
	0.824 a.u. 🔘
For Help, press F1	
	л к
Service X	
Reset error	
Initial Wash - Start	
Tray Move - Front	
Tray Move - Home	
Valve Position Change - Load	
Fill Times Fill Port	
1 V Wash Port 1 V	
Fill Transport Reservoir	
Exchange Needle	
Exchange Syringe	
Exchange Syninge	
Close	

Configuration in Clarity

The appropriate AS 110 control module (under the AS modules) can be selected and configured in the Clarity configuration menu.

王田		Piter: Al		
Name	Status	Vandor	Comment	Module Infa
E 😔 💦				
1100 ALS	installed	Agient	G1313A, G1329A, G1330B a	
360 AS	installed	Konkron	Autoeampler by UNI Ruby scrip	
- 160 AS	initialed	Kontron	Autosianpler by UNI Ruby scrip	
165 AS	installed	Kontron	Autosempler by UNI Ruby scrip	() ()
画 542	installed	ESA		
360 AS	installed	Kontron	Autosampler by UNI Ruby scrip	t
- 193 570 194 580	installed	Grace Attech		
580	installed	Grace Altech		
6850 ALS	installed	Aglent	part of 6850 GC	
- 📑 A2005	installed	CTC		
Told Alien	and allowed	East Haland		Developed by Weiners
- Mas	initialed	Spark Holland	Including User Program	an Manana an Ing
ALS 1000	installed	Deni		
- MOC-20	installed	Shiniedzu	ADC-20, AOC-20s. AOC-20d	
- M AS 100	installed	CVC	Auto Sampler	Testing, Developed by M
A5 100	installed	Antec		
- IN 45 110	installant	Arden.		Developed by Weaver
AS 110 Micro	installed	Antec		Developed by Knauer
AS 3800	installed	Knauer		
AS 3900	installed	Krauer		
- MS 3950	initaled	Kneuer		Developed by Knauer
- M AS 600	installed	PG Instruments		Under development. Der
- 1 AS-1	installed	Knouer	PLATINblue autosampler	Developed by Kneuer
- MS-700	installed	Boom		
- M A52000	installed	Analytical Technologies		
C IT IT TONS	1.+()+.*(*)	+0.00 - 0.00 - 0.00 - 0.00		

Figure 18: Available AS control modules in the Clarity configuration. The AS 110 micro and AS110 standard control module are highlighted in red.

	up Control Modules		Number of Drahumenta: 1
AS Singler 1 GC GC Detector Bolance Thermostat Yalvo Fractos Collector Capitory Hectrophoreis Assilary	Liter	<u>5/N</u> 200301	As induced 1 is induced 2 is induced 5 is induced 6 Instant Type None CC None Attributed 1 Insign for Closed Instrument Insign for Closed Instrument In
			Units Setup Piethod Options

Figure 19: System configuration with AS 110 control module configured and added to LC instrument 1 in Clarity.

Alias Setup		×
Communication	ORS232 OLAN	OUSB
Serial Port	COM1	~
IP Address	192 . 168 . 5 . 2	
USB Port	No port available	\sim
ID	61	AutoDetect
	Status: Unknown	
Sampler Name	Sampler 1	
	Sequence Mode	
	Check Door	
	Enable Alphanumeric Vial Names	
	Digital Input Names	Change
OK	Cancel	Help

Figure 20: In the AS 110 configuration screen LAN communication can be selected and the IP address defined to establish remote communication in Clarity.

To add an AS 110 auto sampler to a LC instrument in Clarity and configure it, please follow the steps below:

- Add the Alias control module to the 'Set-up control modules box in the Clarity configuration window using the 'add' button.
- Double click on the Alias control module to access the set-up menu and set LAN communication and the appropriate IP address.
- Click the 'Autodetect' button to check the communication with the connected AS 110.
- Add the AS 110 to the appropriate LC instrument using the '→' button in the centre of the System configuration menu.
- Set the Ext. Start. Dig. Input to device: "AS 110 (micro) auto sampler" and device: "1", in the case you want to use the digital inject trigger of the auto sampler to start a run in Clarity.
- Another option is to use the analogue start trigger to start a run, in that case a detector or A/D device should be specified as Ext. Start. Dig. Input to start runs in Clarity. For instance the Start input of the DECADE II can be used for this purpose.

After completing these steps the auto sampler is successfully configured as a part of a Clarity LC instrument and can be accessed via the method menu.



In case an ALEXYS analyzer is purchased manual configuration is not required. With these products dedicated instrument installers are delivered with pre-configured hardware settings for Clarity (note AS110 micro is not supported anymore in the instrument installers).

AS 110 method menu

The Clarity method window contains the following AS 110 settings tabs. The most important ones are specified here:

System Settings

Under this tab the following parameters can be set:

- 1. The AS 110 flow path parameters such as loop volume, needle volume and syringe volume.
- The injection method parameters such as syringe speed, Speed scale factor, headspace pressure, needle height, skip missing vials etc. These values affect the standard autosampler injection methods only: full-loop, partial loopfill, uL-pickup. These settings will not apply for the user program.
- Method Setup injection × Select Sampler Cittelabilez Satelier 5 Autosampler Method AS Statut... Injection Wash Inputs & Outputs Mix Liser Program System Spec. Vials Tray Loop Volume [J.L] Syringe Speed Scale Factor 5 Low 10 Syringe Volume (µ) Needle Height 250 🔍 2.0 mm 🗠 Tray Cooling [*C] (active only if available) Wash Transport Position 10 **Fill Port** Pill Volume Wash Volume Reset Outputs 1 1 Wash Port 1 Air Segnent Headspace Pressure (x Syringe Volume) Skip Missing Vials When vial is missing continue sequence Ready, 10 °C From AS Status Measurement Acquisition Integration Calculation Advanced 45 Event Table Send Method Report... ÓK-Cancel Audit Trail..., Help
- 3. Tray cooling: here the tray cooling temperature can be set.

Figure 21: AS 110 system settings tab.

Specify vial and Tray

The specify vial tab allows you to define which trays or well plates are present in the autosampler, tray/well plate processing direction, first destination vial and reagent vial (A-D) positions. The resulting tray configuration is graphically represented under the tray tab.

Method Setup injection					×
Select Sampler	Sarpler 1		Enabled		
	Autosampler	Method		AS Status	
Injection Wash Inputs & Ou	atputs Mix User Program	n System	Spec.Vals Tray		
Plate Type	2.21				
Left 48 vale 🗸	First Destination Vial	OFF	Transport Vials	to OFF	
Right 12 viels 🗸	Reagent Wals				
	A D Relative	OFF			
	8 🔲 Relative	OPP			
	C 🔲 Relative	OFF			
Plate Columna V	D 🔲 Relative	OFF			
Processing Columna ~	(relative range to defai	it start vial -	- 13		
Status Ready, 10	°C			Prom AS	
Event Table AS Measureme	nt Acquisition Integration	Calculation	Advanced		
OK	Cancel Send Me	thod	Report	Audit Tral	Help
Method Setup injection					×
	Alexandra and a second s		s2/////s1		
Select Sangler	Sarple 1		Enabled		
	Autosampler			A5 Status	
Injection Wash Inputs & Ou	/buts Mix User Program	n System :	Spec.Vals Tray		
1 (3) (3) (2) (3) (4)		1	Temp. Start	Val 1 ~	
7 7 15 23 11 3		6 60	Avala	ble	
6 6 14 22 30 3			First C	lestnation	
5 5 13 21 29 3		5 59	Reage	ent A	
	-0-4 Ballion		Reage	nt8	
4 4 12 20 28 3	2 50 5	4 58	Reage	ent C	
3 3 11 19 27 3	5 6	-	Reage	nt D	
2 2 10 18 28 2		3 37	Trans	port	
1 1 9 17 25 3			Error		
ABCDE	P A I	s c			
A B C D E Status Ready.10	S 50 - 6747 - 113	c c		From AS	
	S 50 - 6747 - 113	i c		Prom AS	
Status Ready, 10	rc			Prom AS	
Status Ready, 10	S 50 - 6747 - 113		Advanced	Prom AS	
Status Ready, 10	rc	Calculation	Advanced Report	Prom AS Audit Tral	Help

Figure 22: AS 110 specify vials (top) and tray tab (bottom).

Injection tab

Select Sampler I Sampler I Individ	Method Setup injection				
Injection Wash Inputs & Outputs Mix User Program System Spec. Vials Tray Use User Program (active only if available) Use Timebase Methods Use Siglection Method Injection Method Injection Mode Analysis Time [min.] Nuch Volume [j.L] Needle Volume [j.L] Partial Loopfil 0 30 15 Use Wash Method Wash Between Between Injections	Select Sampler	Sampler 1	2 Empled		
Use User Program (active only if available) Use Timebase Methods Use Sinjection Method Injection Mode Analysis Time [min.] Partial Loopfit 0 0 30 Use Wash Method Wash Between Between Injectors Use Preparative Mode		Autosa	npler Method	AS Status	
□ Use Timetbase Methods □ Use Sigection Method Injection Mode Analysis Time [min.] Partial Loopfil 0 ③ Use Wash Method ☑ Use Preparative Mode	Injection Wash Input	s & Outputs Mix User Pr	ogram System Spec.Vals	Tray	
Use Wash Method Wash Between Between Injectons ~	Use Timebase Method	bi] Plush Volume (jul.) N	ieedie Volume [ul.]	
Wadh Between Between Injectora ↓	Partial Loopfil	- Children (Children)	provide and the second	15	
Use Preparative Mode					
	Between Injections				
Crate Bandy 10 ***	Use Preparative Mode	e.			
Trans, to u.	Status Ready	, 10 °C		From AS	
Event Table 45 Measurement Acquisition Sntegration Calculation Advanced	Event Table AS Mean	arement Acquisition Shlegr	ation Calculation Advance	ed .	
OK Cancel Send Wethod Resort Audit Tral Help	-	Cancel Sw	d Method Derovit	Audit Trail	Help

Figure 23: AS110 control module Injection tab.

Injection tab determines with which injection mode the AS 110 will operate in the method: Full loop, Partial loopfill, μ -Pickup or User program.

The other parameters which can be set under this tab:

- <u>Analysis time</u>: this defines the time between two injection cycles when a series of measurements is programmed in the autosampler
- <u>Flush volume</u>: is the sample pre-flush volume which is used prior to aspiration of sample to fill the sample loop in the full loop and partial loopfill mode.
- <u>Wash:</u> wash between injections or between vials.
- Wash volume: wash volume used for the wash cycle

User program

Under the 'User program' tab a programming interface is available in which fully customized injection methods can be created. Dispense steps, aspirate steps, valve switching, IO actions etc. can be programmed stepby-step in an event table for full flexibility. With this option advanced mixing programs can be created for automated chemical derivatisation / reactions and dilution & mixing.

t Sampler	Sampler 1		Constrained			
		kutosampler Method			AS Status	
vecton Wash Inpu	Its & Outputs Mix	Jeer Program System	Spec.Wals Tra	IY .		
Action				Volume [LL]		
Wash from Valve Wash	Port 1 🗸			200	Add	
1 Weit, time 0.9	0 [min]				Insert	
2 Aspirate Air, #	yringe speed 2, volume 5		af 195.41		Change	
4 Valve, Injector			일이 망명하는 것		Delete	
5 Aspirate from 6 Walt, time 0.0	Sample, syr. speed 2, ne	edie height 2.0 [mm], v	rd. 15 [j.d.]		11150207072110	
7 Valve, Injector					1000000000	
Wash from Val	ve Wash Port 1, volume	200 843			Move Top	
9					Move Up	
					Marce Dinne	
					Photo State State State State	
					News End	
tus Read	fy, 10 °C				From AS	
itTable A5 Mea	surement Acquisition	Integration Calculate	n Advanced			
	and the second second	and and a second				

Figure 24: User program tab.



Note: for every aspirate/dispense step always the syringe speed/height has to be defined.

For more details about the user program steps see chapter 1 paragraph "User program".

Wash tab

Under the wash tab the washing step can be defined.

elect Sampler	Sente 1		Dober		
Signation Wath Sto		itserpie Netfod er Program System	Seer, Hala Trav	AS Stylus	
Single Waith	Treas			(TANCOT	
O Wash Program				Owes	
1 Ginglei Soles				(Dela	
				ment Tat	
				Mine David	
				(Notified)	
	4 , 10 °C			From AS	
saha Rea		nbegration Cokislati	m Advanced	Trom AS	

Figure 25: AS110 control module wash tab.

CHAPTER 4

Using the AS 110

This chapter describes a number of examples of injection actions that can be performed with the AS 110 using the Clarity software. Try to do these examples to learn to work with the AS 110. These examples can be executed after the AS 110 has been successfully installed and after all items described in Chapter 2 and 3 have been correctly set up.

Example 1: 2 µL partial loop fill injection, no wash

The following example is valid for the AS 110 standard, configured with the following items:

- 5 µL sample loop
- 15 µL needle
- 250 µL syringe
- 48-positions sample tray

Log into the Clarity instrument and open the AS 110 method window and enter the settings as displayed in the next screens:

Aethod Setup injection				
select Sampler	Sarpler 1	- Enabled		
	Autosan	opier Method	AS Status	
Injection Wash Input	ts & Outputs Mix User Pro	gram System Spec.Vials Tray		
Use Timebase Metho	-			
Use Injection Method		Flush Volume (µL) Needle Vo		
Injection Mode Partial Loopfil	Analysis Time (min.)	Hush volume (µL) Needle Vo 30 15	une (µL)	
Use Wash Method Wash Between Between Injector				
	ly, 10 °C		Pron AS	
Status Read				
	airement Acquisition Integra	iton Calculation Advanced		

Figure 26: Injection settings under the injection tab.

Under the injection tab set the injection method to 'partial loopfill' and set a sample flush volume of 30 μ L, which is about twice the needle volume.

Aethod Setup injection				
lelect Sampler	Sensier 1	- Dotted		
	Autor	angler Method	AS Status	
Injection Wash Input	is & Outputs Mix User P	rogram Evistem Spec.Vlati Trav		
Loop Volume (LL)	Seringe Speed	Scale Pactor		
5	Low -	-		
Syringe Volume §A.I	Needle Height			
250 -	2.0 mm ~			
They Cooling (*C) (ac	tive only if available)	Wash Transport Position		
12	Reset Outputs	ME Port Fill Volum	e Wash Volume	
W Segment	Interaction pro-	Watibut 1	1	
Sko Meseng Vials	100 000 000 000 000 000 000 000 000 000	(x 5y	inge Volume)	
Date - faith				
When vial is masing	continue pequence			
Status Read	. 9 TC		Fran AS	
	y, a t.		PIGE AG	
IventTable AS Mean	unement Acquisition Sinteg	nation Calculation Advantant		
_				
OK	Cancel Se	nil Nethad Report	Audit Traf	1100

Figure 27: Flow path and aspiration settings under system tab.

riect Sampler	Sever 1		2146						
	Autosmpler	Method				- 10	S Statum	1	
Injection Wash Should & C	utputs His User Program	System	Epec, V	als Trav					
Plate Type	First Destination Viel		Torns	ourt Valu					
Left Histolds V	C Canton	0.66		from	OFF	To	09		
Right #Evials ~	Reagent tiels								
	A 📋 🗌 Ratation	- 1997							
	fi 🔲 Statistice	197							
	C 🔲 Relative	074							
Plate Processing Columns 14	D D Charles	-099							
CONTRACT OF CONTRACTOR	(relative range to defau	a start via	- 10						
	- 10 - 18 - 11 - 1		10						
Status Ready, 8	τ.						From AS		
neady, 81	N						FTER AS		

Figure 28: Tray configuration settings under the specify vials tab.

Click 'Ok' and (re-)save the method file to store all changed settings.

Analysis of the sample can be achieved by programming a sample sequence with a single line. Note that the option "single analysis" in Clarity is intended for injections with a manual sampling valve.

- Make sure that sample tray position, A1 left, contains a sample vial.
- Open a new sequence by clicking the sequence icon in the method window.
- Program one line with SV 1 (start vial) and fill in all other relevant parameters (sample ID, sample, inj. Vol 5 µL, method name etc.).

Click start to execute the sequence.

File	Edit.	Sequ	uence. Vi	ew: Window	e Help 🔣	1	NI K	2 00					
D	-	-	30	n ~ ž	10 m	8	.0	5000	79	a 6) / A @ 5=	β.	
	Status	Run	SV	Sample ID	Sample		Int.vol.	Fie Nane	Std	LH	Method Name	Prest	ľ
2			1:AI	Yest m	Partial loopfil	3	2.000	%q_%R_%3n	Unk		Partial_loopfil	P	

Figure 29: sample sequence for a single injection of 2 μ L sample with partial loopfill method.

Example 2: three full loop injections (5 uL), including wash

In this example three samples are analysed using the full loop injection method. Between every injection a needle wash step is performed.

Aethod Setup Partial_loop	e a la companya de la			
elect Sampler	Sangler 1	< ir frabet		
	Autos	ampler Method	A5 Statut	
Injection Wash Inpu	uts & Outputs Mix User P	rogram System Spec. Viais	Tray	
Use User Program ()		1.] Flush Volume (j.l.) N	eede Volume (j.4.)	
Full Loop	0	30	15	
Use Wash Method Wash Between				
Between Injector	5 V			
Use Preparative Mo	de			
Status Read	ty. 10 *C		From AS	
EventTable AS Mea	surement Acquisition Integ	retion Calculation Advance	d	
	and the second second	and Method Report		Help
0	Cancer 54	Neport	AUGE IF BLUE	Hep

Figure 30: Injection settings in "Mode, Time and Temp." tab.

Set the injection mode to full loop injection. At the wash pull down selector select "wash between injections" and set the wash volume to simple wash 1x under the wash tab. This corresponds to a wash 1x the syringe volume (250 μ L). In the sample sequence editor 3 injections of 5 μ L have to be programmed:

• Make sure that the sample tray positions, A1 to A3 left, contains sample vials.

- Open a new sequence by clicking the sequence icon in the method window.
- Program three lines with SV 1 to 3 (start vial) and fill in all other relevant parameters (sample ID, sample, inj. Vol 10 μ L, method name etc.).
- Click start to execute the sequence.

File	Edit Sequence View Window Help 🔼 🖬 🕅 🖉 💆 😕										
D	실험 성장과 : ~ 사진이 비원님 (今年前日本ア우승 📵 🗡 표종왕 👔										
	Status	Run	SV	Sample 10	Sample	Inj.Vol.	Fie Name	Std	Lyl	Method Name	Prant
1		~	1:A1	Test inj 1	Full loop vial 1	5.000	%q_%R_%31	Unk	1	Full loop	10
2		2 2	1:A2	Testinj 2	Full loop vial 2	5.000	%q_%R_%3n	Unk	1	Full loop	
3	-	4	1:A3	Testing 3	Full loop vial 3	5.000	%q_%R_%31	Unk	6	Full loop	1.0
4	-		1147	TER FUS	FUE KAD YIG 2	3.000	104_104_1031	Con.	-	run wap	10

Figure 31: sample sequence with 3 injections from 3 different vails of 5 μ L sample using a full loop injection method.

CHAPTER 5

Maintenance

For all maintenance procedures:

- Open the door of the AS 110.
- If the cooling option is installed: remove the cooling cover by sliding it towards you.
- Press the two buttons at the top sides of the AS 110 simultaneously and remove the cover by pulling it towards you.



You need not disconnect the AS 110 from the power source for any of the maintenance procedures. In this way software control will still be possible. Use the Direct Control function in the AS 110 Service Manager (ASM) software to check operation of the various parts of the autosampler.

Cleaning

In general, the AS 110 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 "AS 110 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 off.

Injection valve and rotor seal

The AS 110 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. In Direct control, click Initialize to make sure that the valve is in Inject position.
- 10. Perform a standard wash (Direct control Initial wash group box).

The AS 110 is now ready for use.

Sample loop

The AS 110 is standard fitted with a 10 μ L (micro) or 100 μ L sample loop. A different sample loop size can be installed, but note that you will need the proper combination of syringe and tubing (see "Syringe and buffer tubing" on page 13) to ensure good results.

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

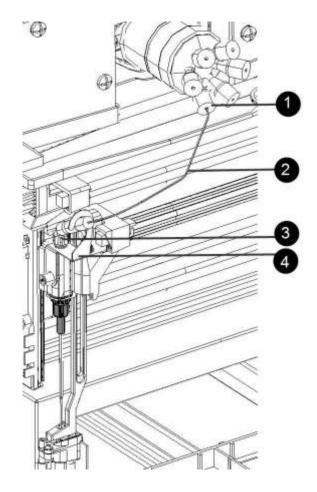
- connect the loop between ports 2 and 5 of the injection valve
- go to the configuration settings and adapt settings in the flow path group box if you have installed a loop with a different volume.

Replacing the sample needle

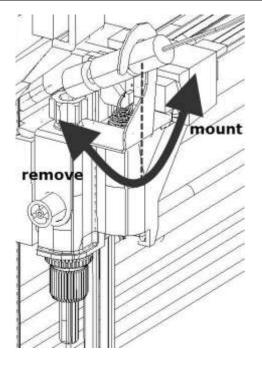


Take great care when replacing the 2.4 μ L micro sample needle: tubing to the needle has a very small internal diameter and overtightening may result in blocked tubing.

Execute the following steps to replace the sample needle:



- 1. Open Direct control (AS 110 Service Manager).
- 2. Click Exchange in the Needle group box. The needle moves to exchange position.
- 3. Loosen the needle connection nut (number 3).



- 1. Turn the needle clockwise to loosen it from its bracket (number 4).
- 2. Loosen the nut (number 1) that connects the tubing (number 2) to port 4 of the injection valve.
- 3. Remove the sample needle by pulling it out of its fitting by the tubing.
- 4. Install a new needle assembly.
- 5. Bend the needle tube towards you and turn the needle counterclockwise to hook it into the needle bracket (number 4).
- 6. Tighten the needle assembly with the needle connection nut.
- 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.
- 8. Click Initialize in Direct control. The sample needle moves back to home position
- 9. Perform a wash routine to clean the new needle by clicking Start in the Initial wash group box of Direct Control.
- 10. Use the Autosampler/Adjustments option to adapt Needle Tray settings.

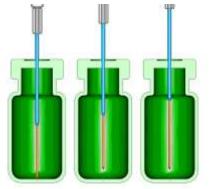


If you use trays with 12 vials or 48 vials, make sure that the needle height settings is > 2mm to prevent the needle from touching the bottom of the vials.

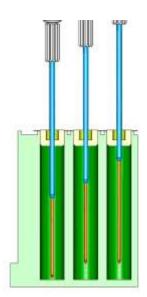
Air needles for AS 110

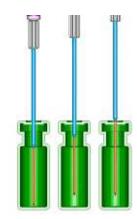
Six types of air needles are available for the AS 110 autosampler, all different in length (difference of 6 mm). These air needles are required to accommodate use of different plate heights in the AS 110. For every well/vial plate the correct air needle is available. Apart from the 6 mm difference in length between the air needle types, the needle holder allows for an extra 6 mm variation in needle height.

Standard air Needle

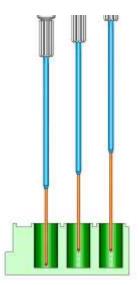


10 mL vial





2 mL vial



Greiner deep well with capmat

Greiner low well

The standard air needle is a 62 mm needle (no. 0045.505). This air needle accommodates use of a wide range of high and low plates. See the illustrations above for the puncturing depth of the needle.

Note that no PASA[™] should be used for low wells: as the sample needle sufficiently punctures the seal to prevent vacuum, the function of the air needle will be insignificant for the low well plates.

If the 10 mL vials are used, the air needle is lowered pretty far into the vial. If the vial is not filled for more than 60%, the air needle can be applied as usual. The same applies for the deep wells.

If you need to deviate from these standard settings, use one of the optional needle types.

Which air needle for which titre plate or vial

To determine which air needle to use, the following dimensions need to be considered:

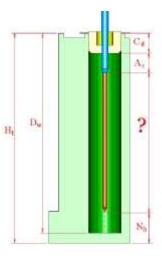
- the height of the titre plate in mm: Ht
- well depth in mm: Dw
- thickness of capmat or seal in mm: Cd
- set needle height in mm: Nh
- distance air needle point through the capmat or seal in mm, min. 2 mm: Ac

The following must be true:

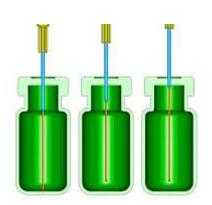
Ht - Dw must be between 2 and 6 mm If this is true, the protrusion length of the sample needle can be calculated; this is the distance between the point of the sample needle and the point of the air needle. It can be calculated as as follows:

Protrusion length = Ht - Cd - Nh - Ac

	Protrusion length		
Air needle type	from	to	
50 mm, yellow	34	40	
56 mm , red	28	34	
62 mm, white (std needle)	22	28	
68 mm, blue	16	22	
74 mm, green	10	16	
80 mm black	4	10	

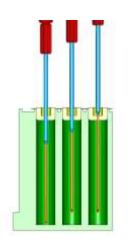


You can select the most suitable air needle on the basis of the protrusion

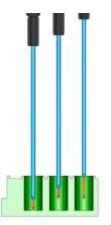


length:

10 mL vial - 50 mm air needle



2 mL vial - 62 mm air needle



Greiner low well - 80 mm air

Greiner deep well M53000, needle with capmat - 56 mm air needle

Example

You have a Greiner deep well with Micronic capmat M53000; the AS 110 has a standard needle height setting. Calculations will be as follows:

Ht = 41.4 mm	The following is true:
Dw = 37.8 mm	41.4 - 37.8 = 3.6 (is between 2 and 6 mm)
Cd = 3.8 mm	Protrusion length = 41.4 - 3.8 - 6.0 - 2.0 = 29.6
Nh = 6.0 mm (standard)	
Ac = 2.0 mm (minimum)	

An air needle of 56 mm is required.

Air needle replacement

Execute the following steps to replace the air needle:

- 1. Remove (see "Replacing the sample needle" on page 29) 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. Get the new air needle.
- 5. 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.
- 6. Install the air needle.
- 7. Install the sample needle.
- Program the proper needle height for the new needle in the ASM settings window. Go to Adjustments to adapt Needle - Tray settings, if necessary.



If you use trays with 12 vials or 48 vials, make sure that the needle height settings is > 2mm to prevent the needle from touching the bottom of the vials..

9. Do an initial wash from Direct control to rinse the needle.

Syringe dispenser

The AS 110 is standard supplied with a 500 μ L syringe, but a 2500 μ L syringe can also be installed for the Prep version. The AS 100 micro is standard supplied with a 25 μ L syringe, but a 50 μ L or 100 μ L syringe can also be installed.

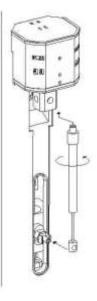


Figure 32: Syringe.

Execute the following steps to install a different syringe:

- 1. In Direct Control, click Exchange in the Syringe group 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.
- Fill the new syringe with wash solvent, preferably IPA (isopropanol). Make sure that most air bubbles are removed from the syringe. In case of a 25 μLsyringe priming with 100% MeOH may be necessary for air bubble free installation.
- 5. Connect the plunger of the filled syringe to the syringe drive and connect the syringe with the connector at the syringe valve.



Insert the end of the plunger well in the plunger lock; if it is not well inserted, the glass syringe will break when the plunger is moving to the home position.

- 6. Screw the syringe firmly into the bottom port of the syringe valve.
- 7. In Direct control, click **Home** in Syringe group 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 **End** again in Direct 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 9 and gently tap the syringe as the wash solvent is dispensed to syringe waste.

Perform a standard wash routine (Direct control: click Start/Stop in de Initial wash group box). All tubing connected to the syringe valve will be refilled and flushed.



Make sure the wash is compatible with IPA, otherwise use a different solvent before switching to the wash solvent.

Syringe plunger & plunger tip

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

- 1. In Direct control, click Exchange in the Syringe group box.
- 2. Remove the syringe.
- 3. Slide the plunger out of the glass part of the syringe.
- 4. With pliers: remove the tip.
- 5. Dampen the new tip with for example isopropanol.
- 6. Mount the new tip on the plunger.
- 7. Insert the plunger in the glass part the syringe.
- 8. Install the syringe 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

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

- 1. Move the syringe to exchange position (use the Exchange button in Direct control).
- 2. Loosen the lower socket-head screw (number 2) a full rotation counter-clockwise.
- 3. Loosen the upper socket head screw (number 1) a full rotation counter-clockwise.
- 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).

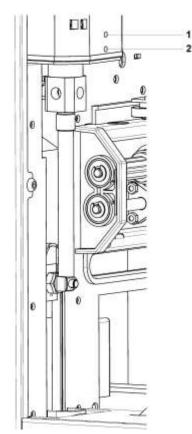


Figure 33: Replacing the syringe dispenser valve.

Fuses

Fuses of the following types are installed in the AS 110: $2 \times 2.5A$



Disconnect the AS 110 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 fuse box at the back of the autosampler.



Contact Service if problems with fuses are recurring.

Needle height adjustment

Program a method in Clarity with partial loop fill or full loop fill method.

tude, Tave and Terra-	Depits and Outputs Mi	Direktro Sampler Method Methods Llaw Progr	1	w 1
Loop Volume (pd)	Tubeg Volume (ul)			
Serrige Volume (ef)	forige lased	Speed Scale Factor	Neede Height (mm)	
E Autopent	E Real Date			
T Ship Meang Viels	12 Headquice Presser	6		
en Ree	d Mode : Not Ready, N	a loaded method		AS Status

In the Clarity Method window on the 'AS/Systems settings' tab, set the needle height to the maximum height (6 mm). Needle height is measured from the bottom of the sample tray to the needle tip when inserted in a vial.

Chapter 5	Maintenance 7
	Program an injection to be performed from an empty vial that is on the
	front row of the sample tray, and run the injection procedure. As soon as
	the needle is inserted in the vial and reached his lowest point, turn off the
	autosampler and inspect the actual needle height in relation to the bottom
	of the vial. Be quick enough to turn off the autosampler, as an actual
	injection of air should be prevented.

Turn on the autosampler again (Clarity may also need to be restarted after the loss of communication). Program a lower needle height, inspect the actual needle height, and repeat until the desired height is found.

The optimal value for needle height can now also be programmed/adjusted in the user program, if applicable.

CHAPTER 6

Trouble shooting

Even though great care was taken in the design of the AS 110, problems may occur:

- *instrument errors:* these can be caused by a variety of reasons.
- *software errors*: usually caused by faulty communication between instruments, or by faulty installation of the software.
- analytical problems (see "Analytical trouble shooting"): 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.

AS 110 Service Manager contains a Service option (select AS 110/Service). 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 AS 110 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. Use AS 110/Direct control in AS 110 Service Manager to monitor the error. Initialize the system in the AS 110/Direct control window.



Make sure the AS 110 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. Select AS 110/Direct control. The Direct control window appears.
- 2. Click Initialize.

- 3. In the Initial wash group box, click **Start** to start the wash.
- 4. Click **Stop** to end the wash.
- 5. Click **Close** to exit the Direct control window.

Execute the following steps if you are asked to initialize the system:

- Select AS 110/Direct control. The Direct control window appears. From this window you can control separate parts of the autosampler to check whether they function as intended.
- 2. Click Initialize to reset the system and prepare it for normal use.

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

- Check that the communication cable between AS 110 and PC is properly installed.
- 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 reinstall 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:

- 1. Check all cable connections between instruments.
- 2. Open AS 110 Service Manager.
- 3. Select the communication port
- 4. Select AS 110/Direct Control.
- 5. Click Initialize.

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.

-	
Causes	Solutions
Air in flow path.	Do an initial wash (select AS 110/Direct
	Control in AS 110 Service 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	Check or replace valve.
valve.	
Rotor seal worn out.	Replace seal. Check stator.
Syringe valve seal	Check if installed
Dead volumes in	Redo connections with new ferrules and
tubing connections.	nuts.

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

Carry over:

Causes	Solutions
Bad match between	Check hardware:
sample characteristics	Needle: either use an extra wash (to
and hardware.	wash the inside and outside needle), or
	install a different type of needle (Steel or
	Silica-coated)
	Valve: replace rotor in valve
	Tubing: install different tubing (Steel,
	Peek) between autosampler and column,
	or use different wash solvents
Sticky sample	Change wash solvents or use multiple
	wash solvents.

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

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

If no injection takes place:

Causes:	Solutio	ons:
Blockage in flow path	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.

The internal diameter of the AS 110 micro		
sample needle is very small and will easily		
get clogged. 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.		
ing and		
C pump.		
w.		
leakage.		
3 and 4:		
check with		

Trouble shooting



Observe the maximum allowed pressure of 350 bar to prevent leakage in the valve! In case of an UHPLC version of the AS 110 the maximum allowable pressure is 680 bar.

CHAPTER 7

I/O Connections

The AS 110 has two I/O connections:

- RS232 connector for serial communication using the SparkLink protocol. Or LAN connector for AS110 autosamplers delivered from 2015 onwards (part number with L)
- Contact closures output and TTL inputs connector.



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.

The IO connector contains active high or active low TTL inputs and one contact closure output, user definable in the System Settings. The two inputs can be programmed as *Next Injection Input, Freeze Input or Stop Input.* The Next Injection Input, Freeze Input and Stop Input can be used to control the AS 110 by other devices.

The contact closure output can be programmed as *Inject Marker, Auxiliary or Alarm output*.

Pin no:	Description:	Cable colors:
1	Output - Common	RED (3-wired)
2	Output - Normally open	BLACK (3-wired)
3	Input 1	RED (4-wired)
4	Input 2	BLACK (4-wired)
5	GND	
6	Output - Normally	BROWN (3-wired)
	closed	
7	GND	
8	GND	ORANGE (4-wired)
9	GND	BROWN (4-wired)

Table 3: IO connector - Contact closure output and TTL inputs

Contact closure output

 Inject Marker Output (default): an Inject marker output will be generated when the injection valve switches from LOAD to INJECT. Status duration of the Inject Marker is the same as for the setting for the SparkLink Inject marker pulse. Range of the adjustment of the inject marker pulse is 0.1 - 2.0 seconds.

- Alarm Output: the Alarm Output will be activated whenever an error occurs, see appendix C for a description of the error codes of the AS 110.
- Auxiliary: the contact closure output can be used as an Auxiliary which can be programmed on a time base up to 4 times On/Off.

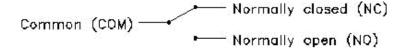


Figure 34: Contact closure output.



Contact closure output: Vmax = 28 Vdc / Vac , Imax = 0.25 ATTL inputs

- Next Injection Input (default): this input will start the next injection sequence After finishing the injection sequence the AS 110 will wait for the Next Injection Input.
- Freeze input: the AS 110 will freeze the analysis time for the time this input is active. If the Freeze Input is activated while the analysis time is not running, the AS 110 will perform all programmed pre-injection sample handling (sample loop). But the AS 110 will wait with injecting the sample until the Freeze Input is no longer active.
- Stop Input: with this input the run of the AS 110 is immediately aborted.

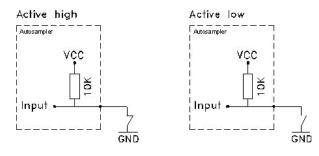


Figure 35: TTL input

CHAPTER 8

Error Codes

The following errors may occur with AS 110:

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.

Electronic

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 AS 110 cannot
	start.

CHAPTER 9

Specifications

General

Sound pressure level	LAeq < 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; cCSAus (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)
Free area around instrument	Minimum free distance of 5 cm from
	obstacles at rear side and air outlets of
	the cooling units.
Weight	19 kg (without cooling)
	21 kg (with cooling)
Max. weight that can be	65 kg
placed on top of AS 110	
Power requirements	95 - 240 Volt AC ± 10%; 50 - 60 Hz;
	200VA
Viscosity range	0.1 - 5 cP

Sampling

Sample capacity	2 Micro Titre Plates according to SBS
	standards; 96-well high/low and 384-well
	low formats, 48-vial or 12-vial trays; any
	combination of plates is allowed, except
	for 384 Low left and 96 High right.
Vial/Plate dimensions (incl.	Max. plate/vial height: 47 mm (incl. septa
cap)	or capmat)
Loop volume	Standard: 1 - 5000 µL programmable, with
	1 µL 10 mL loop optional
	Micro: 0.10 - 20.00 μL programmable with
	0.01µL increments

Dispenser syringe	<u>Standard:</u> 500 μL standard or 2500 μL for
	Prep option
	<u>Micro:</u> 25 μL (standard) or 50 μL and 100
	μL (optional)
Vial detection	Missing vial/well 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
Max pressure rating (valve)	340 bar (HPLC version) , 680 bar
	(UHPLC)
Wash solvent	Integrated wash solvent bottle
Wetted parts in flow path	SS316, PTFE, TEFZEL, VESPEL, Glass,
	Teflon. Optional: PEEK
Injection cycle time	< 60 sec. in all injection modes for 1
	injection \leq 100 µL including 300 µL wash

Analytical performance

Injection modes	Full loop, partial loopfill and µL pickup
	PASA™ (pressure-assisted sample
	aspiration)
Reproducibility AS 110	RSD \leq 0.3% for full loop injections
(500 μL syringe)	RSD \leq 0.5% for partial loopfill injections,
	injection volumes > 10 μL
	RSD \leq 1.0% for μ L pickup injections,
	injection volumes > 10 μL
	(valid at 1.0 cP)
Reproducibility AS 110 micro	RSD \leq 0.3% for full loop injections
(25µL syringe)	RSD \leq 1.0% for partial loopfill injections,
	injection volumes > 0.5 μL
	RSD \leq 2.5% for µL pickup injections,
	injection volumes > 0.5 μL
Carry-over	< 0.05% with 100 μ L needle wash

Programming

Interface	AS 110 Service Manager software
	DataApex Clarity control module
Injection methods	Full loop, partial loopfill and μL pickup
Injection volume	<u>Standard:</u> 1 μL - 5000 μL (with 1 μL
	increment), depending on system settings
	<u><i>Micro:</i></u> 0.10 μL – 20.00 μL (with 0.01 μL
	increment), depending on system settings

Specifications

Max. injection volume	Full loop = loop volume
	Partial loopfill = $\frac{1}{2} \times \text{of loop volume}$ μ L Pick up = (loop volume - 3 x needle
	volume)/2
Injections per vial/well	max. 9 injections
Analysis time	max. 9 hr, 59 min, 59 sec
Wash	Programmable: Wash between injections
	and Wash between vials
Timed events	Programmable: 4 x AUX ON/OFF
Priority sample	Programmable

Communication

Outputs	1 programmable relay output,
	programmable as Inject marker (default),
	Auxiliary, Alarm
Inputs	2 programmable TTL inputs,
	programmable as Next injection input
	(default), Freeze input, Stop input
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)

Options (user-installable)

Bio-compatible sample flow	Inert sample needle (Silco steel) and bio-
path and valve	compatible valve (PEEK)

A P P E N D I X A

Accessories & Spares

The ship kit of the AS 110 contains the following parts:

p/n	Description	Qty
191.0306	Air needle 80 mm (low format well plates)	1
191.0552	CD Rom (ASM en users guide)	1
191.0512	I/O Cable	1
191.0522	Tubing connector T-piece	2
181.0590	power cord EUR	1
181.0592	power cord USA	1
191.0528	Cable Multilink	1
181.0578	Fuse 2,5 AT	2
181.0356	Valco shipkit	1
191.0322	PP wash solvent bottle 250 mL rectangular	1
191.0340*	AS 110 wash bottle adapter	1
191.0342*	AS 110 wash bottle 250 mL, glass	1
191.0328	Silicone tubing (1 meter)	2
191.0300	48 position vial adapter	2

* AS 110 micro is equipped with a glass wash bottle and wash bottle adapter.

The following parts are available for the AS 110:

p/n	description
191.0200	AS 110 accessory kit
191.0200M	AS 110 micro accessory kit
191.0322	Wash bottle 250 mL, PP, rectangular
191.0328	Silicone tubing (1 meter)
191.0340	AS 110 wash bottle adapter
191.0342	AS 110 wash bottle 100 mL, glass
191.0512	I/O Cable
191.0522	Tubing connector T-piece
191.0528	AS 110 serial cable, 9M-9F straight
191.0530	Fuse 2 A
191.0532	Fuse 2.5 A
191.0550	AS 110 Tray cover shell
191.0556	Spacer 2.5 x 2.5 x 30
191.0600	AS 110 vial holder 96 low, start-up kit
191.0602	AS 110 sample vials PP, start-up kit
191.0300	48 position vial adapter
191.0302	12 position vial adapter

Needles		
p/n	description	type
191.0304	Air needle	all
191.0306	Air needle 80 mm	all
191.0314	AS 110 sample needle 15 µL, 1/16"	std
191.0316	AS 110 sample needle, bio, 15 uL, 1/16"	std
191.0314U	AS 110 sample needle 15 μL, 1/32"	UHPLC
191.0315M	AS 110 micro, sample needle, 2.4 µL, 1/16"	micro
191.0314M	AS 110 micro, sample needle, bio, 2.4 µL, 1/16"	micro
191.0315U	AS 110 micro, sample needle, 2.4 µL, 1/32"	UHPLC
191.0332M	AS 110 micro, needle union, 1/32" PEEK	micro/UHPLC

Syringes and plunger tips

p/n	description	type
191.0338M	Sampling syringe 25 µL, ILS	micro/UHPLC
181.0342	Sampling syringe 100 µL, ILS	micro/UHPLC
181.0344	Sampling syringe 250 µL, ILS	std FW >1.26
191.0326	Sampling syringe 500µL, ILS	std
191.0336M	Plunger tip 25 uL (pck/5), ILS	micro/UHPLC
181.0311	Plunger tip 100 μL (pck/5), ILS	micro/UHPLC
181.0543	Plunger tip 250µL (pck/5), ILS	std FW >1.26
191.0313	Plunger tip 500µL (pck/5), ILS	std

Valves, rotor seals and stators

p/n	description	type
191.0330	AS 110 syringe valve	all
181.0324	Valve stainless steel Valco C2-2006	6-p std
181.0332	Rotor seal for Valco C2-2006	6-p std
181.0336	Stator for Valco C2-2006	6-p std
181.0362	Valve stainless steel Valco C2-1006	6-p micro
181.0368	Rotor seal for Valco C2-1006	6-p micro
181.0372	Stator for Valco C2-1006	6-p micro
181.0388*	Rotor seal for Valco C2-1000	10-р
181.0390*	Stator for Valco C2-1000	10-р
181.0392H*	Valve stainless steel Valco C2H-1000	10-р
181.0388H*	Rotor seal for Valco C2H-1000	10-р
181.0390H*	Stator for Valco C2H-1000	10-р
191.0353U	UHPLC valve C82NX-6676	6-p UHPLC
191.0354U	Rotor seal UHPLC valve C72N	6-p UHPLC
191.0356U	Stator UHPLC valve C72NX-6676	6-p UHPLC

*) See warning section on the next page



*) The C2 10p valve is obsolete and is replaced by the C2H 10p valve. The C2H is the improved model, and needs to be purchase as replacement of the C2 valve in case of malfunction. Do not mix parts (stator/rotor) of the C2 and C2H valve, they are not compatible.

Loops		
p/n	description	type
250.1200	Loop 5 ul SS Valco C-type, 1/16"	std/micro
250.1201	Loop 2 uL SS Valco C-type, 1/16"	std/micro
250.1202	Loop 10ul SS Valco C-type, 1/16"	std/micro
250.1204	Loop 20ul SS Valco C-type, 1/16"	std/micro
250.1206	Loop 50ul SS Valco C-type, 1/16"	std/micro
250.1208	Loop 100ul SS Valco C-type, 1/16"	std/micro
250.1214	Loop 1 mL SS Valco, 1/16"	std/micro
250.1220	Loop 1.5 µL SS Valco, 1/32"	UHPLC
250.1222	Loop 2 µL SS Valco, 1/32"	UHPLC
250.1224	Loop 5 µL SS Valco, 1/32"	UHPLC
250.1226	Loop 10 μL SS Valco, 1/32"	UHPLC

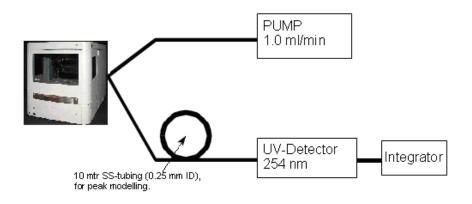
Tubing & connections AS flow path

p/n	description	type
191.0320	Buffertubing 1000µL, Tefzel	std
191.0320M	Buffertubing 200µL, Tefzel	micro
191.0334	AS 110, tubing set	std
191.0334M	AS 110 micro, tubing set	micro
191.0334U	AS 110 micro, tubing set, 1/32"	UHPLC
191.0344	AS 110 syringe valve, nut 1/16"	micro
191.0346	AS 110 syringe valve, flangeless ferrule 1/16	" micro
191.0348	AS 110 syringe valve, nut 1/8"	std
191.0350	AS 110 syringe valve, flangeless ferrule 1/8"	std

A P P E N D I X B

Calibration & Performance

The Autosampler is tested on the analytical performance with the following procedure and test configuration.



Settings

Mobile phase and wash solve	ent :	H2O
Volume of installed loop	:	100 µL
Syringe volume	:	500 µL
Syringe speed		normal
Needle-valve tubing volume	:	15 µL
Buffer tubing	:	1000 µL
Tray type	:	High 96 well
Head space pressure		no
Air segment	:	no

Method

Test #1	μL pick-up
Injection volume:	10µL
Wash:	Between injections
Wash volume:	500µL
Analysis time:	01:00
Sample	50 ppm uracil
Injections/well:	3
First sample:	A1
Last sampler:	A5

Test #2	Partial loopfill
Injection volume:	10µL
Flush volume:	45µL
Wash:	Between injections
Analysis time:	01:00
Sample:	50 and 1000ppm uracil
Blank:	Mobile phase (H ₂ 0)
Injections/well	3
First sample:	A6
Last sample:	A12

Fill A1 till A10 with 50 ppm Uracil. Fill A11 with 1000 ppm Uracil, and A12 with H2O. Determine for μ L pick-up the RSD (Chrom Perfect) and determine the RSD and carry over of the partial loopfill injection by:

$$RSD\% = \frac{\sigma_{n-1}}{Peak \ area} \times 100 \ \% \qquad Carry \ over = \frac{Peak \ area \ blank \times 5}{Peak \ area \ 50 \ ppm} \ \%$$

The calculated RSD and carry over must be within the following specs:

Reproducibility	- RSD ≤ 1.0% for µl pick-up
	injections
	- RSD \leq 0.5% for partial loopfill
	injections
Carry over	< 0.05% with programmable needle
	wash

N.B. The final test is programmed in AS 110 Service Manager (ASM). Via service and validation / life test, there can be chosen between 4 experiments under validation test. Those are:

- #1: µL pickup,
- # 2: partial loopfill
- # 3: full loop
- # 4: prep mode

By choosing the experiment and clicking on start, the final test will be automatically processed.

APPENDIX C

Maintenance injection valve

The AS 110 is standard equipped with a Valco C2-2006 injection valve. The AS 110 micro with either a Valco C2-1006 or C2-1000 injection valve. The AS 110 micro, cool UHPLC is equipped with a Valco C72NX-6676 injection valve Cleaning a valve can often be accomplished by flushing all lines with appropriate solvents. With normal use the valve will give many tens of thousands of cycles without trouble. The main cause of early failure, which is seen as a leak in the valve, is abrasive particles in the sample and/or mobile phase, which can scratch the rotor seal. Following is the procedure for changing the rotor seal.



NOTE: Do not disassemble the valve unless system malfunction is definitely isolated to the valve. UHPLC valves should not be opened at all and serviced by authorized service personnel of the manufacturer.

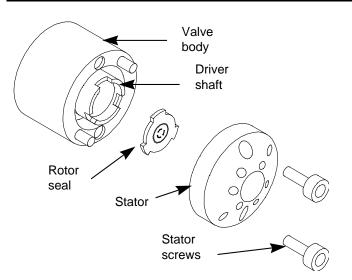


Figure 36. Exploded view of injection valve.

Disassembly

- 1. Use a 9/16 hex driver to remove the socket head screws which secure the cap on the valve.
- 2. To insure that the sealing surface of the cap is not damaged, rest it on the outer face. Or, if the tubing is still connected, leave it suspended by the tubing.
- 3. With your fingers or small tool, gently pry the rotor away from the driver.

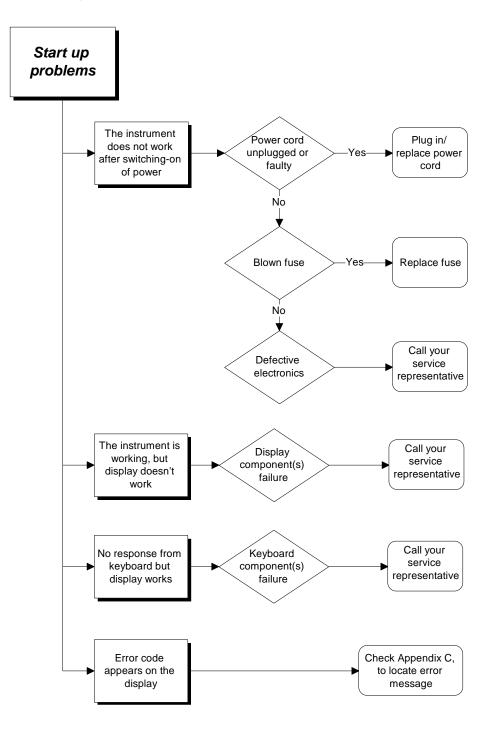
4. Examine the rotor sealing surface for scratches. If scratches are visible to the naked eye, the rotor must be replaced. If no scratches are visible, clean all the parts thoroughly with an appropriate solvent, taking care that no surfaces get scratched. (The most common problem in HPLC is the formation of buffer crystals, which are usually water-soluble) It is not necessary to dry the rotor.

Reassembly

- 1. Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The pattern is asymmetrical to prevent improper placement.
- 2. Replace the cap. Insert the two socket head screws and tighten them gently until both are snug. *Do not over-tighten them* the screws simply hold the assembly together and do not affect sealing force, which is automatically set as the screws close the cap against the valve body.
- 3. Test the valve by pressurizing the system. If it does not hold pressure, the valve should be returned to Valco for repair.

APPENDIXD

Trouble shooting



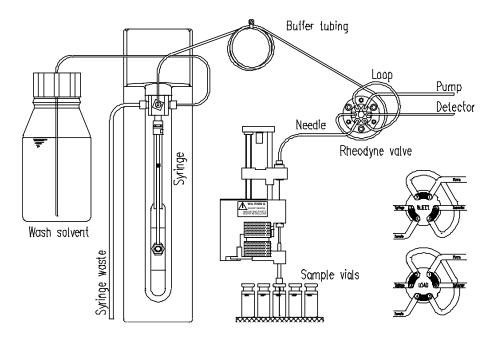
Analytical problems

In case of analytical problems you will have to determine whether they are caused by the autosampler or by the rest of the system.



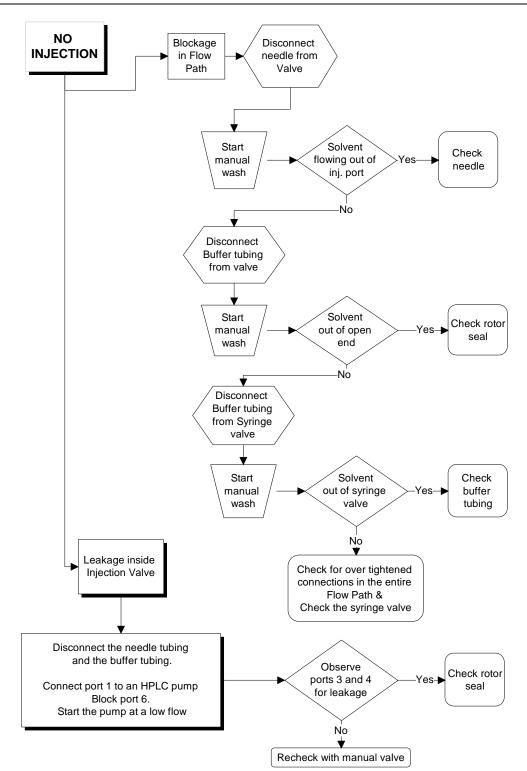
Quick check!

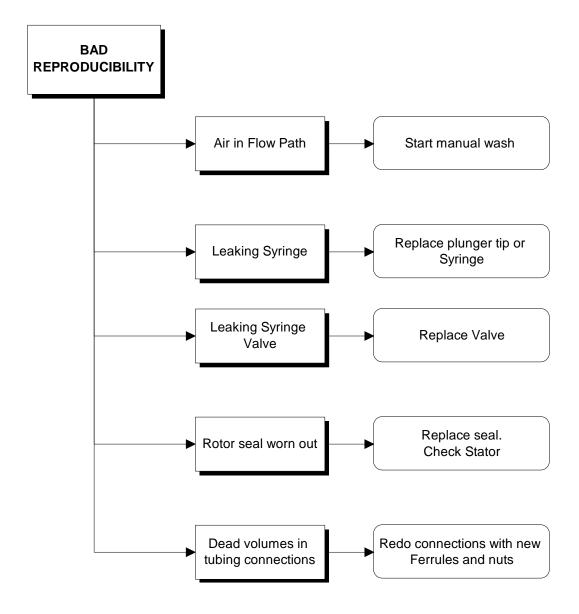
Replace the valve by a manual injection valve to discriminate between valve problems and other problems. Perform a number of manual Flushed loop injections. If the results are good, the autosampler is faulty; if not, the HPLC system should be checked.





In the flowcharts on the next pages it is assumed that the AS 110 does not display any error messages. Please keep in mind that analytical problems might be caused by external influences, like temperature and/or lightsensitive samples. For this reason it is important to make sure that the application was running without problems before and that no changes have been made in the settings (System Menu).





APPENDIXE

Storage, packaging, recycling and disposal

If the autosampler needs to be stored for a long time, or if it must be shipped to a different location, proceed as follows:

- 1. Thoroughly flush the system.
- 2. Disconnect and remove all tubing, except for the sample loop.
- 3. Switch off the autosampler.
- 4. In case of biohazard materials: remove and throw away the needle and other flow path materials, in accordance with an approved waste disposal program.
- 5. Use the original packaging materials to package the autosampler; also place the foam block in the tray location.
- 6. Fill in a health & safety form for the instrument and include the form in the package.
- 7. Dispatch the package; make sure any tax/import/export requirements are met.

Contact support@antecscientific.com if you need more information.



Decontamination

The instrument shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

General instructions for disposal

When taking the instrument out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Hazardous substances

The instrument contains hazardous substances. Contact the manufacturer for more detailed information.

Disposal of electrical components



Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

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