PAL SYSTEM

Prep and Load Platform

Tips and Hints for PAL Systems including general technical HPLC information

1. Valve Type and Characteristics

Valve P/N	No. of Ports Valve Type Inlet Port	Valve Bore Size in mm	Tube Connect. in inch	Stator P/N Material	Rotor P/N Material	Flow Rate Range	Remarks
C2V-3006-CTC-K	6 C Vertical port	0.75	1/16	C2V-3C06 sst	C2-30R6 Valcon H	5 to 100 mL/min	Preparative application Bore size requires needle Gauge 19 Max. pressure 5000 psi/340 bar
DC6WK-K	6 W Vertical port	0.40	1/16	na sst	SSAC6W Valcon H	0.5 to 5.0 mL/min	Valve for standard HPLC application. Column ID 4 mm, flow 1 mL/min Max. pressure 5000 psi/340 bar
C2V-2346-CTC-K	6 C Vertical port	0.40	1/16	C2V-2C46 PAEK	C2-23R6 Valcon E	0.5 to 5.0 mL/min	Biocompatible Valve for standard HPLC flow rates Max. pressure 5000 psi/340 bar
C2V-1006D-CTC-K	6 C Vertical port	0.25	1/16	C2V-1C06 sst	C2-10R6 Valcon H	10 to 500 µL/min	Valve for semi-micro columns ID 1 and 2 mm Max. pressure 5000 psi/340 bar
C2V-1346D-CTC-K	6 C Vertical port	0.25	1/16	C2V-1C46 PAEK	C2-13R6 Valcon E	10 to 500 µL/min	Biocompatible Valve for semi-micro columns, ID 1 and 2 mm Max. pressure 5000 psi/340 bar
C2V-0006D-CTC-K	6 C Vertical port	0.15	1/16	C2V-0C06 sst	C2-00R6 Valcon H	100 nL/min to 100 µL/min	Injection Valve for micro columns Vertical port bore 0.25 mm, reduced to 0.15 mm at bottom Max. pressure 5000 psi/340 bar
C2V-0346D-CTC-K	6 C Vertical port	0.15	1/16	C2V-0C46 PAEK	C2-03R6 Valcon E	100 nL/min to 100 µL/min	Biocompatible Injection Valve for micro columns. Vertical port bore 0.25 mm, reduced to 0.15 mm at bottom. Max. pressure 5000 psi/340 bar
CN2-4346D-CTC	6 C No vertical port	0.10	1/32	CN2-4C46I PAEK	CN2-43R6 Valcon E	10 nL/min to 10 µL/min	Biocompatible Valve for column switching only. Stator sst. wetted parts PAEK
C72VX-1696D-CTC-K	6 C Vertical port	0.25	1/16	C72V-1C96 N60C	C72-16R6 Valcon E3	10 to 500 µL/min	Valve for ultra-high pressure use Max. pressure 15 000 psi/1030 bar
C72VX-6696D-CTC-K	6 C Vertical port	0.15	1/16	C72V-6C96 N60C	C72-66R6 Valcon E3	100 nL/min to 100 µL/min	Valve for ultra high pressure use Max. pressure 15 000 psi/1030 bar
PD7991	6 R Vertical port	0.20	1/16	7991-005 Ti-plated with SPC-1	7991-999 PEEK blend RPC-10	10 to 500 µL/min	Valve for ultra high pressure use Vertical port bore 0.25 mm, reduced to 0.20 mm at bottom. Max. pressure 15 000 psi /1030 bar

Listing is not complete. Only most common valve types listed. Other configurations, such as 10-port or internal loop valves, are available.

Key and Remarks to the Table "Valve Type and Characteristics":

P/N:
- "CTC": Specific modifications for CTC Analytics AG.
- "-K": indicates a kit containing nuts, ferrules, Needle Guide, Needle Seals and Teflon waste tubing.

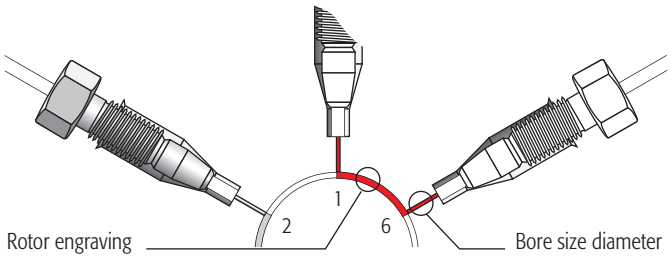
Valve type:
- W-Type: Conical rotary valve, VICI/Valco.
- C = Cheminert-type : Flat plate rotary valve, VICI/Valco
  Plumbing for two different valve types is shown below.
- R = Rheodyne, flat plate rotary valve.

Bore Size: Valve connection port inlet/outlet path diameter.
See graphic at right.

Stator: sst = Stainless steel, N60 (corresponds to SS316)
N60C Stator coated for UHPLC use.
PAEK polymer related to well-known PEEK material.

Rotor material:
- Valcon H: Reinforced carbon fiber composite PTFE lubricated, inert engineered polymer. Standard material from VICI/Valco.
- Valcon E: Polyarylether ketone/Teflon (PAEK/Teflon) material if biocompatibility is required.
- Valcon T: Polyimide (Vespel)/PTFE/carbon composite. A choice when high temperature is required.
- Valcon E3: Wear resistant coating for UHPLC (proprietary, VICI/Valco).

For material-specific information and limitations consult VICI/Valco product information bulletin.



2. Valve Type, Bore Size versus Valve Volume

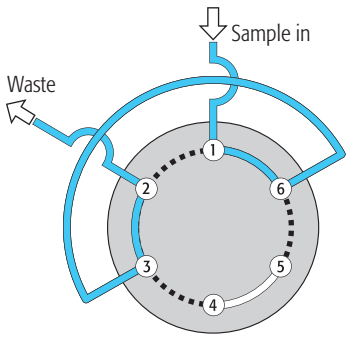
Valve volume specification:
Loop overfill (full loop): 2 ports and 1 engraving
Partial loop filling: 1 port and 1 engraving.

Sample loss at injection, Valve contribution (not overfill volume)
Loop overfill (full loop): Content of 2 ports and 1 engraving
Partial loop filling: Content of 1 port, engraving is injected.

Valve Bore Size and Valve Type	Volume for Port, Engraving			Valve Volume for Partial Loop	Valve Volume for Loop Overfill
0.75 mm Cheminert, sst	Port: 1 Engraving:	2725 nL 1173 nL	Other ports: 2725 nL	6623 nL	3898 nL
0.40 mm W-Type, sst	Port: 1 Engraving:	155 nL 390 nL	Other ports: 155 nL	545 nL	700 nL
0.40 mm Cheminert, sst	Port: 1 Engraving:	615 nL 170 nl	Other ports: 220 nL	1005 nL	785 nL
0.40 mm Cheminert, PAEK	Port: 1 Engraving:	650 nL 170 nL	Other ports: 345 nL	1165 nL	820 nL
0.25 mm Cheminert, sst	Port: 1 Engraving:	320 nL 70 nL	Other ports: 75 nL	465 nL	390 nL
0.25 mm Cheminert, PAEK	Port: 1 Engraving:	290 nL 70 nL	Other ports: 130 nL	490 nL	360 nL
0.15 mm Micro Cheminert, sst	Port: 1 Engraving:	273 nL 40 nL	Other ports: 30 nL	343 nL	313 nL
0.15 mm Micro Cheminiert, PAEK	Port: 1 Engraving:	514 nL 40 nL	Other ports: 40 nL	594 nL	554 nL
0.10 mm Nano Cheminert, PAEK	Port: 1 Engraving:	8 nL 13 nL	Other ports: 8 nL	29 nL	21 nL

All values are theoretical values. Values may change without notice and are provided by VICI/Valco.
Rheodyne PD 7991: Port to port volume: 195 nL, 294 nL for vertical port (Port 1).

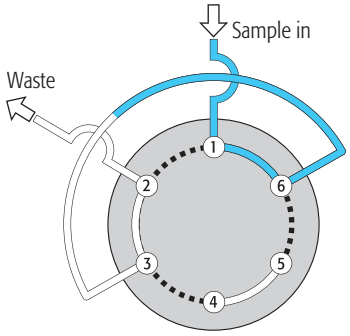
3. General Rules for Loop Filling



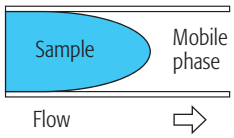
Full loop injection:
Overfill Loop 3 to 5 times.

Example 1: Small loop size: Loop 20 µL. Volume needed to fill loop 60 to 100 µL.

Example 2: Large loop size: Loop 200 µL. Use at least 300 µL to fill the loop



Graphic A



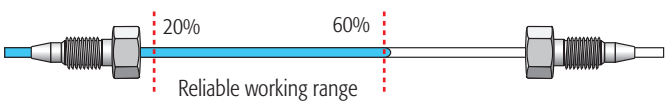
Partial loop filling:
Reliable working range: 20 to 60% of loop content.

Example: Small loop size: Loop 20 µL. 4 to 12 µL sample volume.

Loops with larger volume can be filled within a range of 20 to max. 80% of loop content (200 µL or larger).

Disregarding the rules will result in poor repeatability.

The reason for these rules is the principle of hydrodynamic flow patterns in the solvent front reaching the loop inlet and outlet. *See graphic A above.*



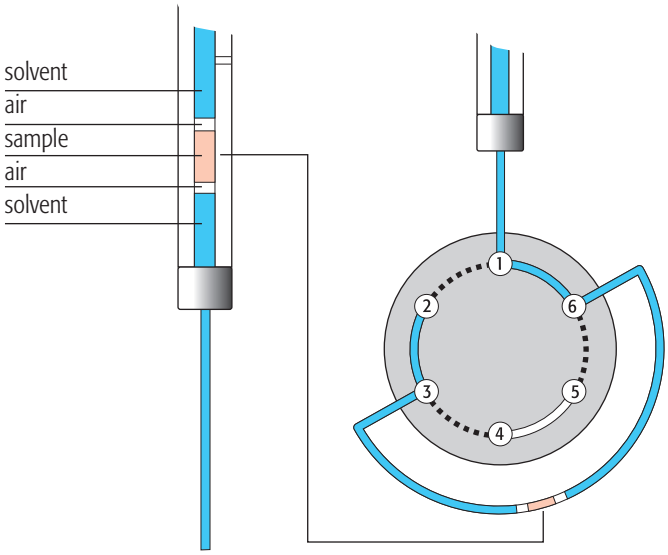
Injection speed:
Example for a 20 µL loop: Injection speed is 5 to 10 µL/s.
Higher speed will cause turbulence in the loop, resulting in poor repeatability.
Injection speed is a PAL method parameter and must be adjusted for the type of solvent (viscosity and boiling point), loop size, or rather loop internal diameter, and the valve bore size.
See the recommended method parameters listed in the PAL firmware software overview.

4. Low Volume Pick-up

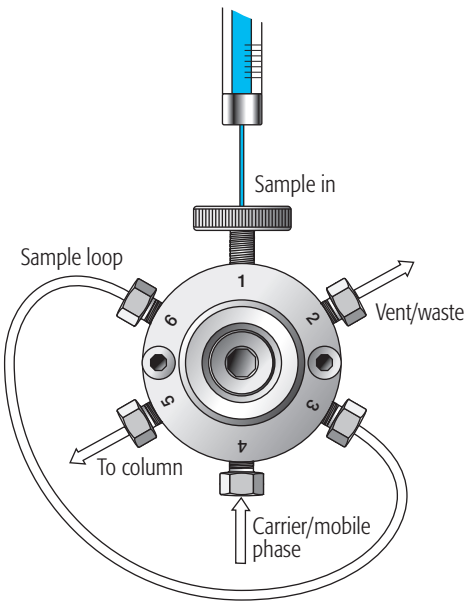
If a limited amount of sample solution is available, use of "low volume pick-up" mode is recommended. The sample solution is embedded in a solvent sandwich separated by small air gaps. The sample plug is positioned mid-loop.

Important:
Use a solvent with a lower elution power than the starting composition of the mobile phase gradient.

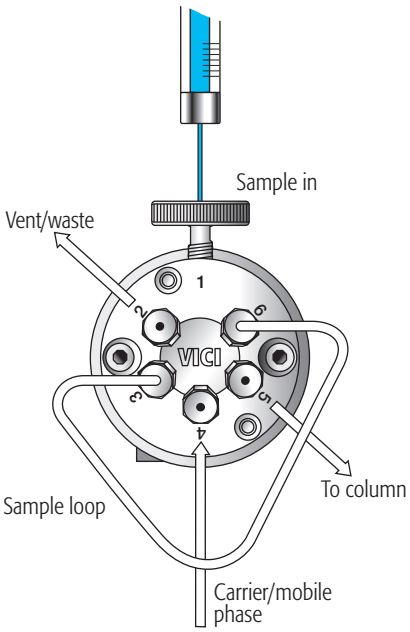
A sample macro for the Cycle Composer software is available from any CTC Analytics representative. The macro is written with built-in flexibility to adapt to syringe, loop, and sample volumes.



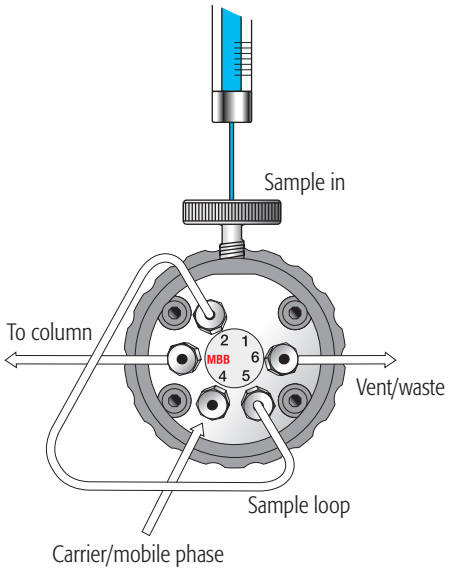
5. Plumbing Diagram for the W-and Cheminert- Valve Types



VICI/Valco W-Type Valve
Conical rotary valves
(No biocompatible version available)



VICI/Valco Cheminert Type Valve
Flat plate rotary valve
(Biocompatible version available)



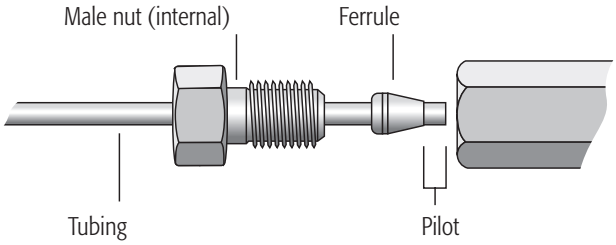
Rheodyne PD7991-K
15 000 psi /1030 bar Flat plate rotary valve
Rheodyne Make-Before-Break® (MBB) technology features reduced pressure transients during valve actuation

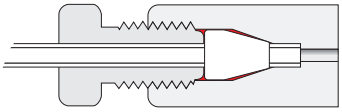
6. Nuts and Ferrules

- Do not use a nut or a ferrule from a vendor other than specified for the product.
- While tightening the nut, keep tubing tightly positioned to ensure correct pilot distance.
- Do not over-tighten the nut / ferrule.
- Do not reuse an installed nut / ferrule for any other connection.
- Eliminate trapped air by installing nut / ferrule in wetted ports only.

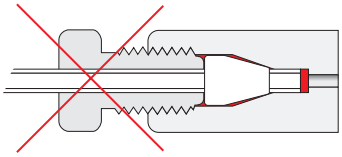
Disregarding these basic rules will lead to:

- Dead volume
- Peak deformation or peak splitting
- Carry-over effect

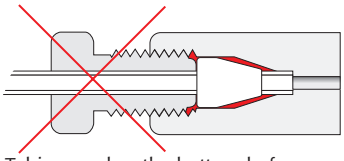




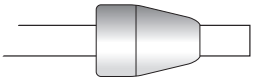
Tubing seats correctly at the bottom



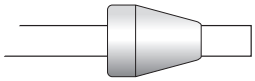
Tubing doesn't reach the bottom, introducing dead volume



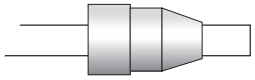
Tubing reaches the bottom before ferrule seats



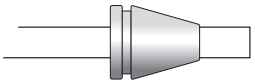
Valco



Parker



Rheodyne



Swagelok

7. Tubing Internal Diameter versus Flow Rate

The tubing internal diameter must be adjusted to flow rate, valve type, and application to avoid high backpressure or chromatographic irregularities.

Tubing ID
Points to consider are:

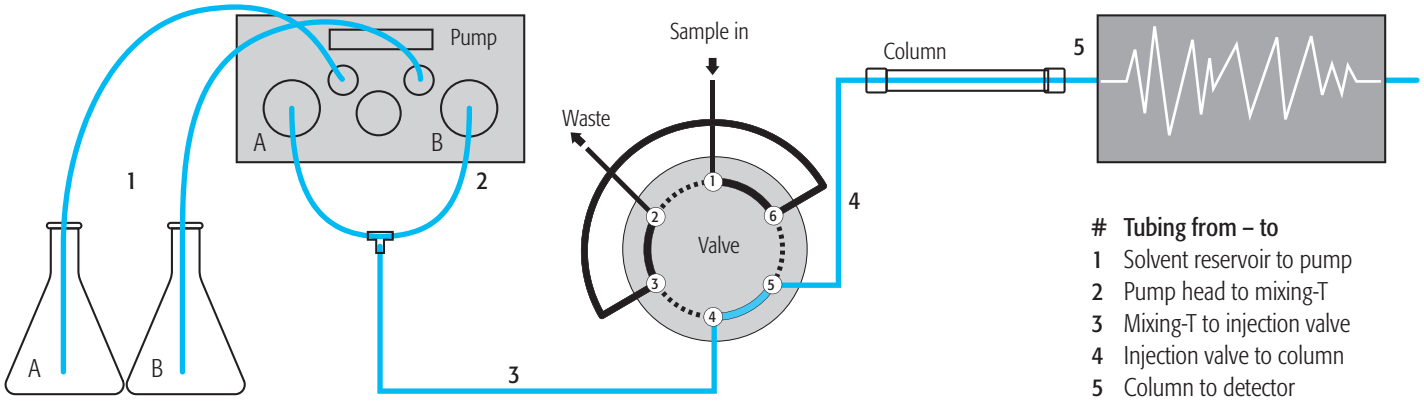
- Delay volume of entire HPLC System
- Time needed for gradient to go active at column inlet
- Adjust tubing diameters and length:
  - Solvent reservoir to pump: Cavitation?
  - Pump head to mixing-T: Low backpressure
  - Mixing-T to injection valve: Low backpressure; as short as possible
  - Valve to column: ID as small as possible, considering backpressure
  - Column to detector: ID as small as possible (if possible, smaller than # 4 considering backpressure)

Tubing Internal Diameter versus Tubing Volume
Examples are calculated for a tube length of 100 mm.

Tubing inch	Tubing in mm	Tubing volume
0.040	1.00	78.55 µL
0.020	0.50	19.64 µL
0.010	0.25	4.91 µL
0.005	0.13	1.33 µL
0.0025	0.064	0.32 µL

Valve Type Bore Size in mm	Flow Rate Range from – to	Tubing ID inch / mm	Tubing from – to # see diagram below
0.75 mm	5 to 100 mL/min Preparative application	0.25" / 6.35 mm 0.040" / 1.0 mm	1: Reservoir 2–5: Same ID for entire HPLC sytem plumbing to avoid backpressure
0.40 mm	0.5 to 5 mL/min Standard HPLC Column ID 4 mm	0.125" / 3.18 mm 0.020" / 0.50 mm 0.010" / 0.25 mm	1: Reservoir 2–3: Pump to Valve 4–5: Valve to Detector
0.25 mm	10 to 500 µL/min Standard HPLC Column ID 1 to 2 mm	0.040" / 1.0 mm 0.010" / 0.25 mm 0.005" / 0.13 mm	1: Reservoir 2–3: Pump to Valve 4–5: Valve to Detector
0.15 mm	100 nL to 100 µL/min Micro flow application	0.020" / 0.50 mm 0.020" / 0.50 mm 0.005" / 0.13 mm 0.005" / 0.13 mm	1: Reservoir 2: Pump Head to Mixing –T 3: Mixing-T to Valve 4–5: Valve to Detector
0.10 mm	10 nL to 10 µL/min Nano flow application Fused silica 50 µm	0.020" / 0.50 mm Fused silica 50 µm 3–5: Fused silica 25 µm	1: Reservoir 2: Pump Head to Mixing –T Mixing-T to Detector flow rates > 1 µL/min 3–5: Mixing-T to Detector flow rates < 1 µL/min

Data for Tubing IDs are recommendations only. Variations depend on the application, mobile phase, flow rate, column ID and sample load.



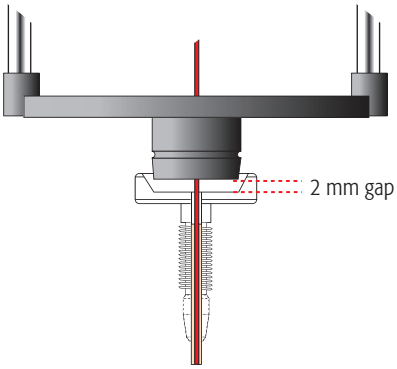
 # Tubing from – to

1 Solvent reservoir to pump
2 Pump head to mixing-T
3 Mixing-T to injection valve
4 Injection valve to column
5 Column to detector

8. Needle Guide and Needle Seals

Positioning of Injection Unit on Valve Needle Guide

Position Injection Unit approx. 2 mm above bottom of Valve Needle Guide.

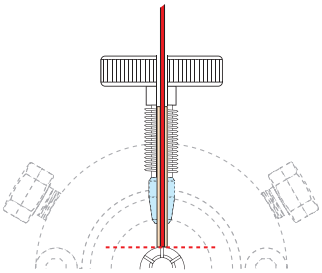


Effect if Injection Unit is not positioned correctly

- Loss of steps from Z-stepper motor. (PAL will recover steps at point of axis-referencing.)
- Possibility that next object in cycle will not be detected (if next step follows directly without axis-referencing. Example: Wash Station)

Needle Seal and Needle Guide

- Change the Needle Seal at regular intervals
- Do not use steel ferrules for a PAEK valve stator
- Select the appropriate Needle Seal/Guide as listed in the table below

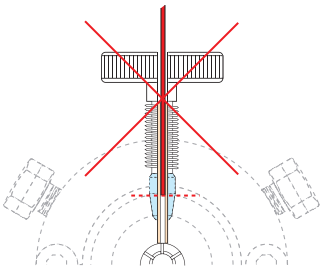


Effect if Needle Seal is not tight

- Loss of sample or no sample transfer to valve
- Filling injection port (Port 1) with sample solution
- Carry-over

Needle Penetration in Valve Inlet Port

- Path: Menu/Utilities/Injectors/F3 – move to injector
- Activate "Needle Penetration"
- Move needle down slowly until a clearly audible click occurs
- Motor pressure release 2 steps up
- Check position again
  Correct position shown in graphic (Needle Seal



Needle Penetration in wrong position

- If needle is too high: No seal possible. Carry-over
- If needle is too low: Needle tip hits top of valve body. Possible needle distortion

Needle Guide and Seals replacement information

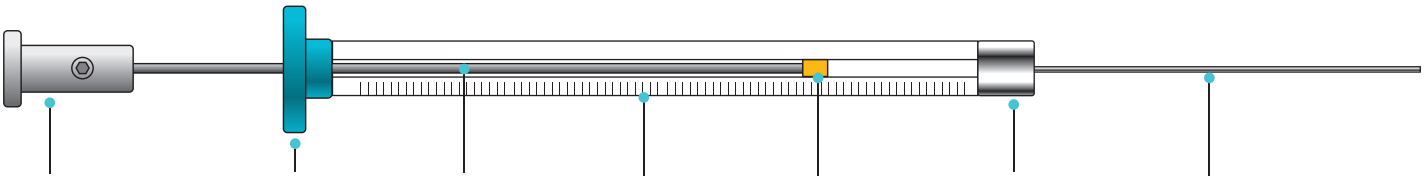
P/N	Description	Remarks
PAL NdlSeal	Needle Seal Gauge 22	Transparent FEP tubing with sst ferrule
PAL NdlSealP	Needle Seal for PAEK Valve Gauge 22	PEEK ferrule to protect PAEK valve stator Transparent FEP tubing
PAL NdlSealR	Needle Seal 22 Rheodyne	Transparent FEP tubing with sst Rheodyne ferrule
PAL NdlSeal-19	Needle Seal Gauge 19	Blue colored Teflon tubing to differentiate from Gauge 22, sst. ferrule
MV 30-12	Needle Guide for Gauge 22 Needles	sst material
MV 30-30	Needle Guide for Gauge 22 Needles	PEEK material for PAEK valve
MV 30-52	Needle Guide for Gauge 19 Needles	Groove in rim to differentiate from Gauge 22 type. sst. material

9. Syringes and Needles

CTC Analytics ***X***-Type Syringe developed for:

- High throughput applications
- Biological samples/drug discovery/proteomics
- Practically zero carry-over

E***X***tra long life and E***X***tra low carry-over
No metal contact
Inert, smooth surface



Adjustable plunger, protects plunger tip from being squeezed

Color coded flange indicating needle internal diameter

Plunger stem sealed with temperature and solvent-stable material

Glass barrel inner surface polished and sealed for inertness. Stable to organic solvents, acids and bases, or brine

New inert plunger tip polymer with significantly longer lifetime

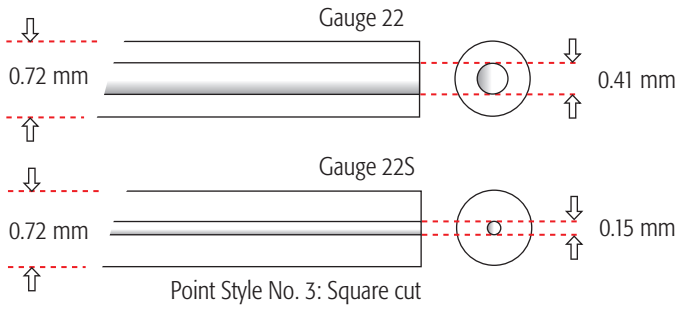
Needle fixation to barrel. No sample contact with glue or cement

Deactivated needle with extremely smooth inorganic glass layer prevents metal contact. Stable to organic solvents, acids and bases, or brine

Syringe max. vol.	CTC	Hamilton		Syringe description			
ml	Article No.	P/N	Description	Glass OD mm	Gauge	Point style (PST)	Remarks
	X-Type syringes, SyrX - for high throughput and biocompatible applications						
25	SyrX G25-22S-3		1702 CTC (22S/3) inert	7.7	22S	3	Metal flange: red color
50	SyrX G50-22S-3		1702 CTC (22S/3)inert	7.7	22S	3	Metal flange: red color
100	SyrX G100-22S-3		1710 CTC (22S/3) inert	6.6	22S	3	Metal flange: red color
100	SyrX G100-22-3		1710 CTC (22/3) inert	6.6	22	3	Metal flange: blue color
	C-Type syringes, SyrC, - standard HPLC syringes						
10	SyrC G10-22S-3	203194	1701 CTC (22S/3)	6.6	22S	3	Metal flange
25	SyrC G25-22S-3	203075	1702 CTC (22S/3)	7.7	22S	3	Metal flange
100	SyrC G100-22S-3	203077	1710 CTC (22S/3)	6.6	22S	3	Metal flange
100	SyrC G100-22-3	203235	1710 CTC (22/3)	6.6	22	3	Metal flange
250	SyrC G250-22-3	203079	1725 CTC (22/3)	7.7	22	3	Metal flange
500	SyrC G500-22-3	203349	1750 CTC (22/3)	7.7	22	3	Metal flange
1000	SyrC G1000-22-3	203081	1001 CTC (22/3)	7.7	22	3	Glass flange
2500	SyrC G2500-22-3	203083	1002 CTC (22/3)	9.5	22	3	Glass flange
5000	SyrC G5000-22-3	203085	1005 CTC (22/3)	13.5	22	3	Glass flange

G = Gastight/ Plunger Teflon. | Plunger Tip for ***X***-Line: Teflon/Polymer mix | Replacement plungers are available at CTC.
X-Type Syringes are sold exclusively by CTC. Flange color indicates needle internal diameter. Example: Gauge 22 blue or Gauge 22S red.

Syringe Needles / Standard Needle for HPLC Technique:



Needle Gauge 19: OD 1.04 mm. Mandatory for prep valve with bore size 0.75 mm. (Needle with Gauge 22 fits into valve bore.)

Needle gauge versus fill speed and Needle volume

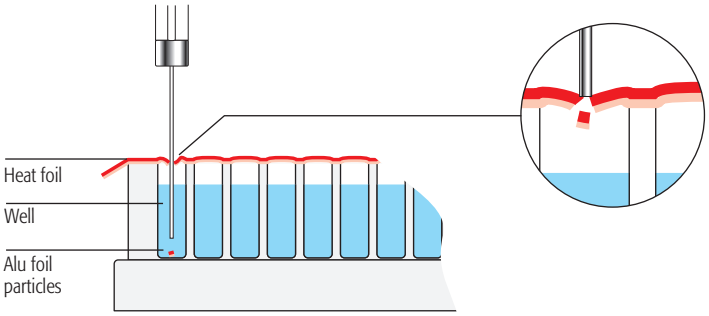
Needle gauge	Fill speed[1])	Time to fill a syringe	Needle volume[2])
Gauge 22S	5 to 10 µL/s	10 to 20 s	0.90 µL
Gauge 22	200 µL/s	0.5 s	6.73 µL

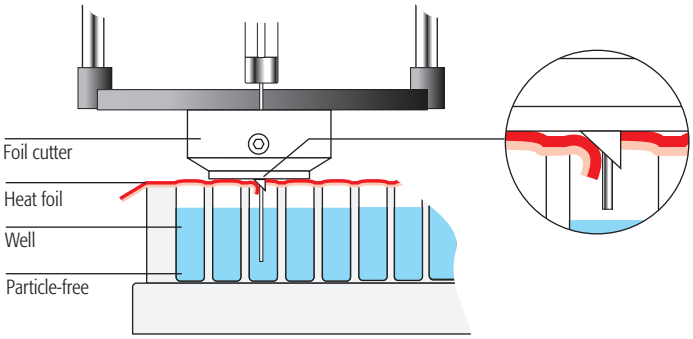
Example: 100 µL syringe

[1]) Maximum fill speed before cavitations are observed.
Fill speed example with solvent water / methanol (1:1)

[2]) Needle volume for 51 mm standard needle

10. Microtiter / Deepwell Plates and the Piercing of Foils





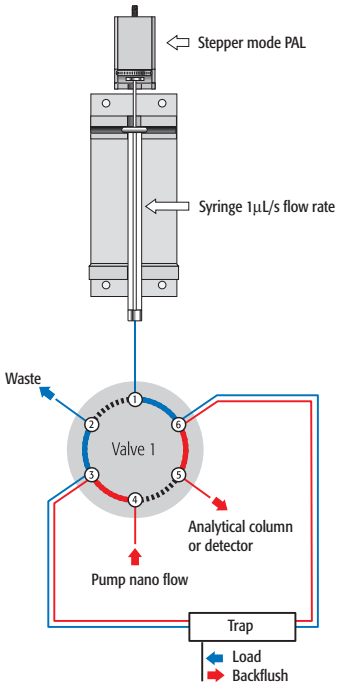
- Foil cutter: Article No. MSZ 02-01
- Foil cutter can be used for all types of heat foils
- Do not use the adhesive seal foils
  Organic solvents, such as DMSO, can dissolve glue

11. Examples of Plumbing Diagrams for Micro/Nano Flow Applications

- Transferring sample in µL range easier to handle than in nL range.
- Transfer sample first to a trap to focus with a "high flow rate".
- Use partial loop filling to minimize loss of sample.
- Adjust sample and wash solvent to chromatographic strength of mobile phase.
- Use a backflush mode to optimize transfer from trap to next device.
- First wash step for biological samples must be with aqueous medium followed by organic solvent.
  Last wash step must be with aqueous medium (achieved with a "pre-wash" directly before next injection).
- Trap can be used for sample preparation.
  Example:
  - Eliminate buffer/salt or high molecular weight compounds.
  - Solid Phase Extraction (SPE)
  - Restricted Access Material (RAM)
  - Molecular Imprint Polymer (MIP)

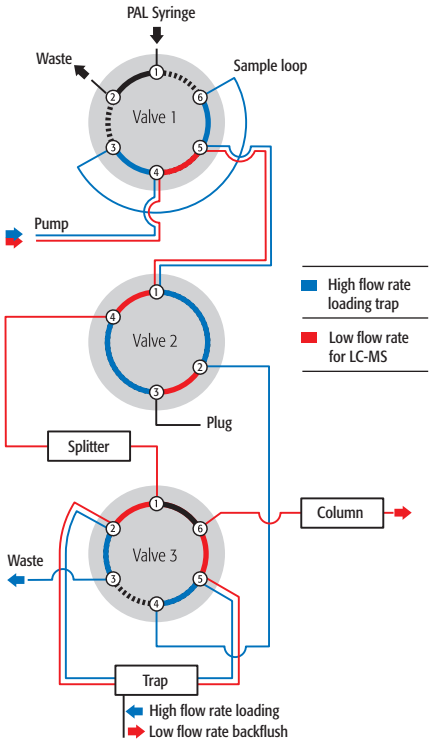
PAL System used as a Syringe Pump

- Loop is replaced with a cartridge or trap.
- PAL System used as a Syringe Pump to load the cartridge or trap.
- Slow deposition of the sample solution, 1 mL/s.
- Advantage: Simple setup. Only one valve and one HPLC pump required.



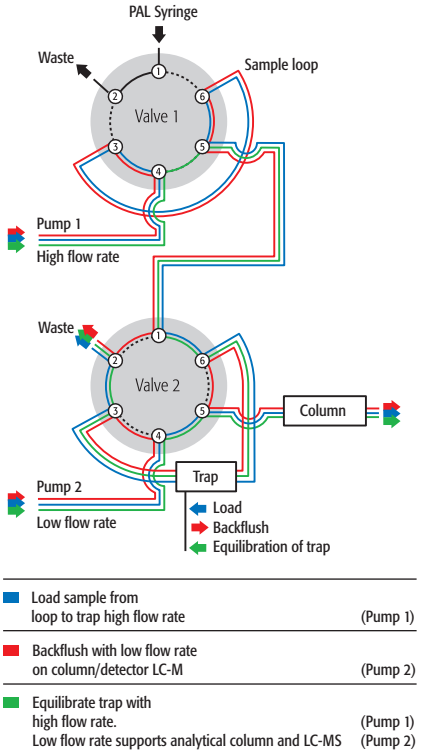
Valve System, Single Pump with Splitter

- Sample transfer with high flow rate.
- Control of the analytical flow rate with the splitter device after valve switches.
- Place splitter as close as possible to column.
- Advantage: One pump system required.
- Disadvantage: 3-valve setup. Splitter must work reliably.



Valve System and Two-pump Setup

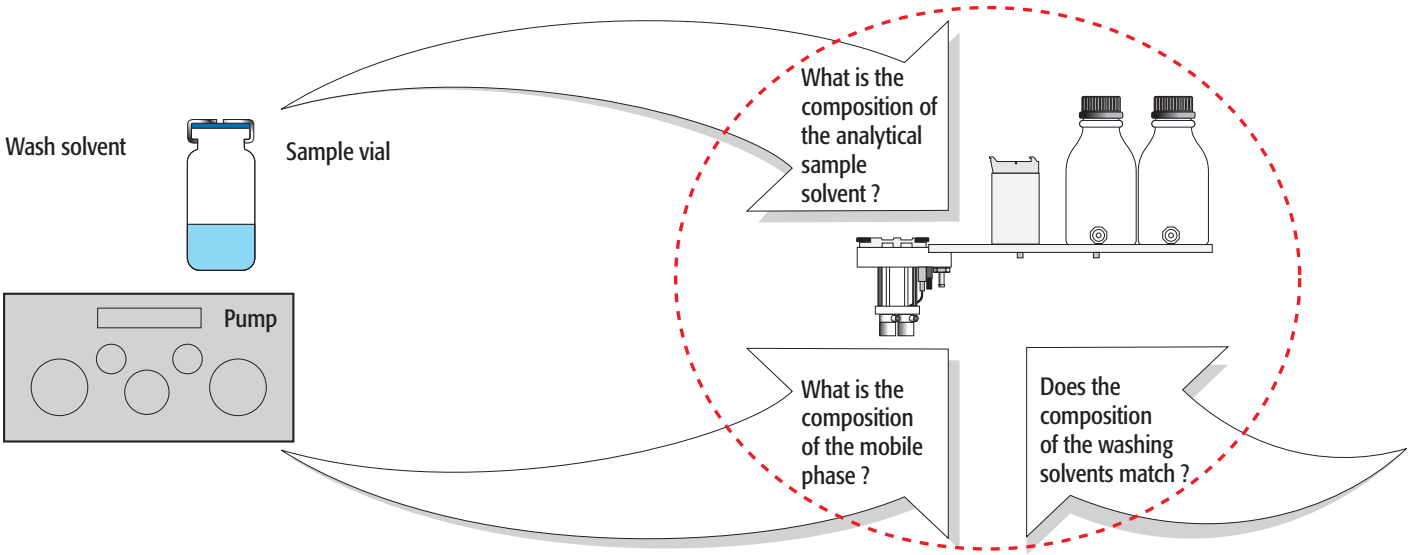
- Two pumps are operated independently of each other.
  High flow rate for fast sample transfer on trap.
  Low flow rate optimized for LC-MS application.
- Mobile phase from first pump reconditions the trap.
- Advantage: Clean design with ease of troubleshooting.
  Two dedicated flow ranges (pumps).
- Disadvantage: Two pumps required.



Load sample from loop to trap high flow rate	(Pump 1)
Backflush with low flow rate on column/detector LC-M	(Pump 2)
Equilibrate trap with high flow rate.	(Pump 1)
Low flow rate supports analytical column and LC-MS	(Pump 2)

12. Carry-over | Troubleshooting

Carry-over and repeatability



Wash steps for biological samples:

- 1st Wash cycle: Aqueous solvent
- 2nd Wash cycle: Organic solvent.
- 1st Wash cyle before next sample:
  Pre-wash with aqueous solvent
  (Eliminate organic solvents in syringe and valve).

No.	Important points to check	Influence on carry-over	Influence on repeatability	Remarks
1	**Wash solvents:** Does the wash solvent match?	!	!	*See Point 12 above*
	Wash solvent in Loop for next injection: Is the chromatographic strength adjusted to the Mobile Phase?		!	Avoid organic solvents with high elution potential remaining in loop
2	**Valve system!** Is the valve intact?	!	!	Are any leaks observed?
	Is the waste line open?	!	!	At loop fill, observe movement and speed of solvent front.
	Is the backpressure at normal level?	!	!	
	Can the valve be activated?	!	!	Menu/Utilities/Injectors/LC Vlv (F1)
	Is the Valve Type (dimensions) appropriate for the flow rate?	!	!	*See Point 1*
3	**Needle seal:** Is the seal tight?	!	!	Does the seal fit in size? Gauge 22/19? *See Point 8*
4	**Needle penetration:** Is needle penetration in valve inlet port adjusted?	!	!	Is the Injection Unit needle guide (Z-Axis) correctly positioned? *See Point 8*
5	**Nuts and ferrules:** Are only matched nuts and ferrules used?	!	!	Are the tubings cut square and are they open without any restriction?
	Are all connections remade (dead volume)?	!	!	*See Point 6*
6	**Loop injection:** Full loop injection: Is the loop overfilled 3 to 5 times?		!	*See Point 3*
	Partial loop filling: Is the rule of 20 to 60% of the loop content applied?		!	Consider also the valve volume as indicated in *Point 2*
7	**Syringe:** Is the plunger tight? Is the syringe intact?	!	!	
	Is a change to the ***X***-Type syringe indicated?	!	!	High throughput applications? High affinity of a compound for metal?
8	**Material replacement:** Tubing: sst to PEEK or fused silica?	!	!	Compare flow rate vs. tubing ID. *See No. 7, directly above.*
	Valve rotor: Is a change from Valcon H to Valcon E indicated?	!	!	*See Point 1*
	Valve body: Is a change from sst to PEEK indicated?	!	!	Observe material advantages and limitations. Product information is available from supplier.
9	**PAL method parameters:** Syringe fill speed: Cavitation?		!	Gauge 22S or 22? *See Point 9*
	Pullup delay: Is the time long enough?		!	
	Injection speed: Is the loop fill speed too high?	!	!	For recommendations see the PAL firmware overview or the PAL User Manual.
	Eject speed: Is the syringe eject speed high enough?		!	Eject speed is a syringe parameter. Air bubbles are ejected during fill strokes.
10	**HPLC column:** Is the inlet frit clean/replaced?	!	!	HPLC System backpressure?
11	**HPLC system parameters:** Column and trap Is the selected equilibration time long enough?		!	General rule is to flush the column/trap 5 to 10 times with the starting condition of the gradient
	Is column equilibration timed correctly?			
12	**Detection and integration:** Is the peak detection and integration verified?		!	Peak tailing? Baseline assignment? S/N ratio? Area rejected? Peak slope detection?
	Is the signal within the dynamic linear range of the detector?		!	Linearity?

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