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HTSL – 1100
Protasis High Throughput Sample Loader: Technical Notes (FEP Tubing Version)

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Overall Procedure

The High-Throughput Sample Loader (HTSL-1100) will arrive plumbed and, once primed with the solvent of choice, it is ready for use.

The function of the HTSL-1100 is quite simple. It delivers solvent at a specified flow rate, stops at an indicated time or volume, then refills. The plumbing configuration allows the user to load a sample loop prior to a run, and the sample loop contents are delivered to the CapNMR™ probe, and flow is stopped.

The capillary inserted into the orange sleeve that connects to the pump was positioned to not protrude past the inside end of the sleeve. The capillary position is limited in motion at the entrance of the sleeve with a small piece of adhesive tape. Should you have occasion to change this connection, *be sure to re-assemble it in the same manner*.

Begin by priming the pump with the solvent of choice; we use D_2O as the solvent for our standard calibration. Priming is most effectively performed when the pump is in the empty position. In the Protyle™ software, under the instrument control tab, there is a pump empty command. When priming is complete, power on the HTSL. It is best to initially not connect to the probe when setting up the HTSL for the first time. See the detailed priming procedure for a complete description.

Flush the HTSL plumbing by setting the stop volume to a fairly large value, such as 90 μL, and perform an injection command. A suggested delivery rate is 5 μL/min and a refill rate of 25 μL/min. Do this at least twice to ensure good rinsing. It is not necessary to load the loop with a sample. You should observe fluid emerging from the capillary outlet that goes to the probe. A refill value of 25 μL/min is a reasonable maximum for the refill rate.

Connect the NMR probe using a P-779-01 union and fittings, and flush the probe with solvent by again performing an injection command. Again, it is not necessary to load the loop with a sample. If you overpressure the pump ($P > 2000$ psi), it will stall (see LED key). Just re-power, and choose a lower flow rate or shorter tubing for the rinsing steps.

The NMR probe should be locked on D_2O and shimmed.

Load the loop (3 μL; 1.5 m) with a sample of 14 mM acetone-p_6 in D_2O. Use the 10-μL syringe, and push the plunger very slowly at a rate of approximately 0.5 μL/sec. Set the stop volume to a value that makes the pump run for about 8 min (40 μL at 5 μL/min). This depends somewhat on the length of tubing used: 2 μL per meter of 50 μm i.d. tubing. Make sure you initially set the stop volume to a volume large enough to move the sample into and out of the NMR probe. The probe volume is about 8 μL total, including attached capillary.

Meanwhile, set up NMR data acquisition to a take a single-scan spectrum every 6 sec. Note that this is every 0.1 min, or every 0.5 μL. A great way to do this on Varian consoles is to set d1 = 6 – at. Also, array nt = 1, but let the array increment equal zero. If you type in ga, then dssh, this will display the spectra side-by-side as they are acquired. You have to re-type dssh frequently to update the spectra. The idea is simply to create an NMR time base that begins when injection begins, that is, when the HTSL starts pumping. You might set this up to acquire 80 spectra, that is, 8 min of data. You may begin pumping and NMR acquisition simultaneously, or use a known offset, such as beginning data acquisition at t_{pump} = 12 sec. This way, the first NMR spectrum is acquired and processed at t_{pump} = 18 sec. The time offset between the pump and the NMR spectra must be considered for correct calibration.

Perform an injection command on the HTSL and simultaneously begin acquiring NMR data. Note that the first spectrum is acquired at t = 6 sec, not at t = 0.

You will see the acetone go through the NMR flow cell. The acetone signal is located at about 2.2 ppm in the solvent.

Compute the time at which the acetone maximum occurs in the NMR data.

Enter into the HTSL the volume corresponding to the computed time of the signal maximum.

Reload an acetone sample into the loop.

Initiate an injection and begin acquisition of NMR data simultaneously, or using a known offset. The acetone will appear in the NMR flow cell, the flow will stop, and the NMR signal will level off. The NMR acquisition can be aborted, and other NMR data acquisition experiments can then be performed. The sample will remain parked in the NMR flow cell for as long as the user desires.

A different sample can be made in the solvent, loaded, parked, and NMR data acquired.

If a long NMR acquisition is desired, plug the outlet capillary of the probe with an extra P-779-01 union and tan PEEK plug (#P-550) to ensure long-term (greater than 1 hour) sample stability.

The HTSL is 4" high, 14.5" wide, and 10.5" deep.

An extension cable can be added to the 3-pin cable used to connect the HTSL to a PC. It is prudent to still include the 3-pin cable as a segment in the connection. A good source for extension DB9 cables is:

www.cablesnmor.com/mouse-extension-cable.html

6-ft	#P22122
10-ft	#P22123
25-ft	#P22124

LED Key

The front panel of the HTSL has three LEDs:

Yellow:	Loading (Deliver)
Green:	Ready
Blue:	Power

Normal Operations

Emptying after power-up, or during a flush	Yellow and Green LEDs blink alternately
Filling after power-up, or during a flush	Yellow LED blinking Green Steady on
Ready (pump full)	Yellow LED off Green LED steady on
Delivery	Yellow LED steady on Green LED off
Refill	Yellow LED blinking Green LED off

Error Indicators

Valve 1 problem	Yellow and Green flash together once, then pause and repeat; homing failure
Valve 2 problem	Yellow and Green flash together twice, then pause and repeat; homing failure
Pump problem	Yellow and Green flash together three times, then pause and repeat; homing failure or stall

Detailed Priming Procedure

Begin with the HTSL completely plumbed with the desired tubing. For the manual loading configuration, all of the tubing is 50/360 (50 μm i.d., 360 μm o.d.), except for the fluid path from the solvent reservoir to Valve 1 to the pump head, and from Valve 2, Port 5 to the waste port, which is 150/360. For the Gilson configuration, the flow path from the loading port connection to V2P6 is also 150/360. Do not connect the outlet capillary (V2P3) to the probe quite yet.

The HTSL should be set in advance to a Fill and Delivery rate of 25 μL/min. Power on the HTSL. Watch for the LED panel to switch from emptying (yellow and green LEDs blink alternately), to filling (yellow LED blinking). The entire process takes about 8.5 min. Once the Ready light is on, and the pump is idle, connect the PC program to the HTSL. Proceed to the Instrument Control tab, and hit "Pump Empty". Wait about 4 min for this command to complete. The pump is then in prime position with V1 set to connect to the solvent reservoir. Keep in mind the pump volume is 100 μL.

Fill a 1-mL, Teflon Luer lock, air-tight, glass syringe with the deuterated solvent of interest. It may be advantageous if the solvent is first sonicated and blanketed under helium, however, this does not appear to be necessary. Attach the fitting (Upchurch #F-120) on one end of a 3-inch segment of FEP tubing (cut with nice, square tips using a razor), 1/16" o.d., and 0.030" i.d. (Upchurch #1522). Attach the F-120 to the syringe with an adapter (Upchurch #P-659). Fit the other end of the short tube with an F-287 black PEEK nut and ferrule and screw it tightly into the side (upward) port of the pump head. Tighten snugly with a ¼" wrench.

Push firmly on the syringe and verify that fluid is discharged from the capillary tip which is normally placed in the solvent reservoir (normally a fluid inlet). Discard about 400 μL of this fluid, and don't discharge it into the solvent reservoir. Once this is done, place that same capillary tip into the reservoir containing additional solvent, and push about 100 μL out into the container with the capillary tip submerged. Next, draw up the fluid, about 200 μL per pull, and wait about 1 min between pulls. If the pump is completely cleared of bubbles, you should be able to pull up 200 μL or more without the appearance of a bubble. Look carefully at the translucent FEP tube to confirm the appearance or lack of a bubble in the flow path. Initially, the bubbles that appear are actually air inside the syringe pump, and will not collapse within the syringe barrel. However, if you pull quickly, bubbles form from cavitation, that is, a negative pressure within the syringe barrel. Some bubbles will originate from cavitation, which you should allow to collapse by influx of fluid.

Once you have stopped pulling up bubbles, make sure the air bubble in the (vertically positioned) syringe is at the top of the barrel. Once the FEP tube is filled with fluid, wait at least 15 min before proceeding. Then, while holding the syringe vertically, push firmly down on the plunger, and confirm that no bubbles appear at the capillary tip inside the solvent reservoir, but be careful not to push air back into the pump. If no bubbles appear in the solvent reservoir, unscrew the syringe from the P-659 PEEK adapter. Loosen it completely and keep the FEP tubing, still filled with solvent, in a vertical position. Next, gradually loosen the F-287 fitting at the syringe side port, and allow the solvent in the FEP tube to drain into the hole. Leaving that fluid in place, remove the F-287 fitting and screw the plug (Upchurch #P-550) into place in the side port. Tighten firmly with the plastic wrench (Upchurch #P-298).

Next, using the Instrument Control tab again, hit "Pump Fill" and wait about 4 min. While still not fluidically connected to the probe, check that the Fill and Delivery rates are both still set to 25 μL/min. Set the Load Volume to 75 μL, save it, and perform a Run. You should see fluid emerge from the outlet capillary which leads to the probe (Valve 2, Port 3). Should you overpressure the pump (P > 2000 psi), it will stall (see LED key); just re-power, and choose a lower flow rate or shorter tubing length for the cycling steps. If the trial run completes successfully, and without any leaks detected, proceed to perform rinsing cycles. First, attach the transit capillary to the HTSL outlet capillary (V2P3). This will insure that priming runs are performed at high pressure to increase the likelihood of bubble removal. Perform another, single trial Run with the Delivery rate set to 10-15 μL/min. If that trial runs completes successfully, set the number of cycles to 5 (Instrument Control tab), and press Empty/Fill. Verify that fluid again emerges from the outlet capillary. Allow the HTSL to complete this rinsing step of about 60 min.

Once these cyclic runs are complete, the HTSL should be in a primed state. With a 3 – 4 meter section of capillary connected to Port 3, Valve 2, and the Delivery Rate set to 5 μL/min, fluid should appear at the capillary outlet tip within ~30 sec of commencing a Run command of at least 20 μL of Delivery Volume. Next, perform a run (F = 5 μL/min; D = 90 μL; which is 18 min delivery time) while fluidically connected to the probe. The probe should be completely empty (flushed with acetone then air) before being rinsed with solvent. Note that the sample loop need not be loaded for any of the rinsing steps, as it is filled with solvent at all times.

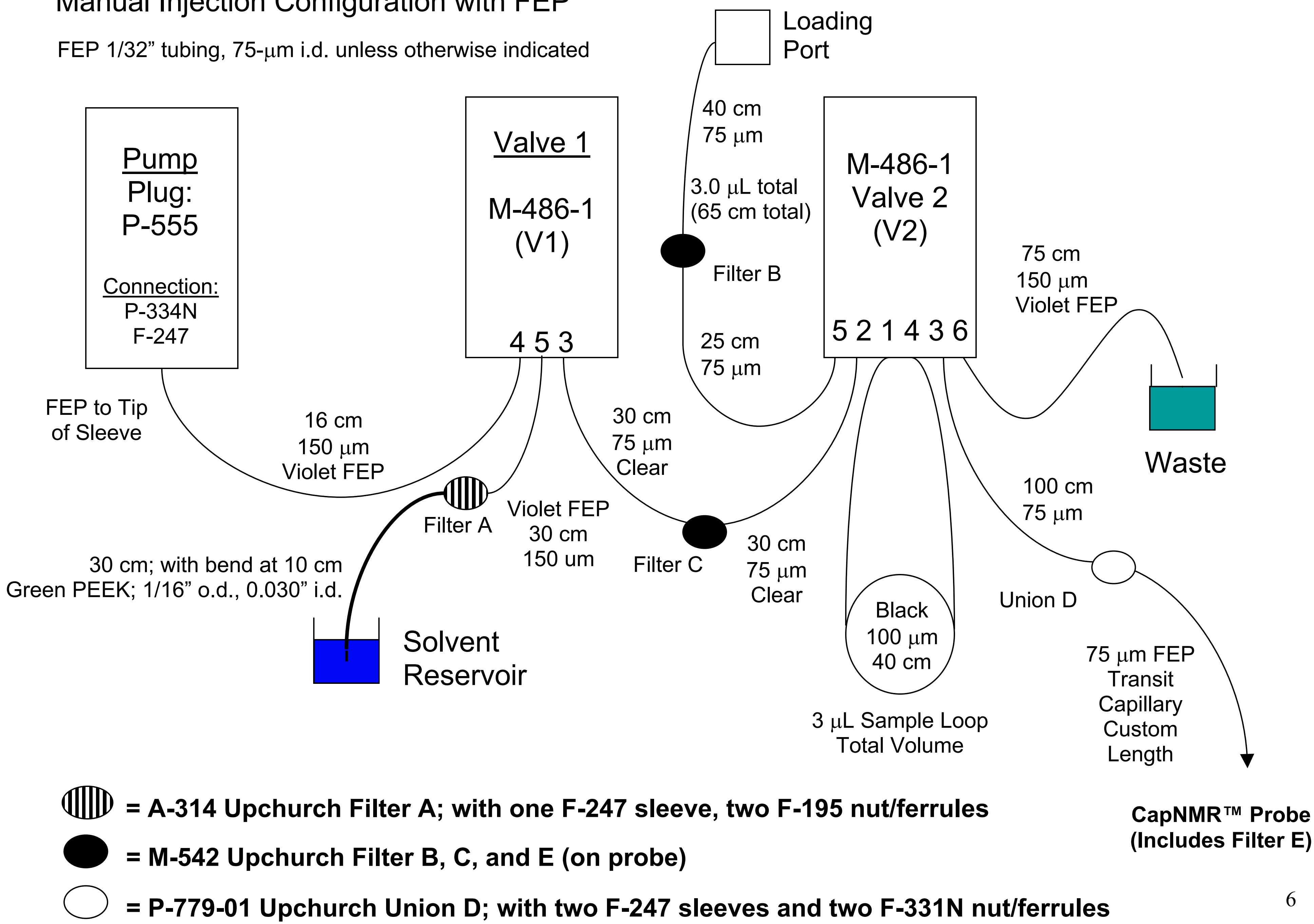
To verify priming, repeatedly load and inject a sample of the deuterated solvent spiked with protonated acetone (or any other suitable sample). A 1:1000 dilution of acetone in the solvent makes a nice sample of about 15 mM acetone-p_6. Load the loop slowly with the 10-μL syringe to ensure proper filling of the loop. Be sure to only load the loop when the pump LED indicates "Ready", and the HTSL is idle. Try an initial load volume of 50 μL, which is intentionally larger than that actual volume from sample loop to NMR probe. With a 1-scan NMR spectrum acquired every 6 sec, the maximum of the side-by-side spectra should appear in the same spectrum from injection to injection. If the reproducibility is close, but not perfect, perform another 90-μL run with the probe connected. Re-check the reproducibility, and if unacceptable, you will need to re-prime.

The key to priming success seems to be patience. It might take 3 hours to properly prime, so you should probably do it while doing something else. The pump should stay primed for an indefinitely long period, as long as the capillary fluid inlet tip is kept submerged in the reservoir solvent. If the HTSL fluid is changed to a different solvent, you will need to rinse the pump with the new solvent, then re-prime as before.

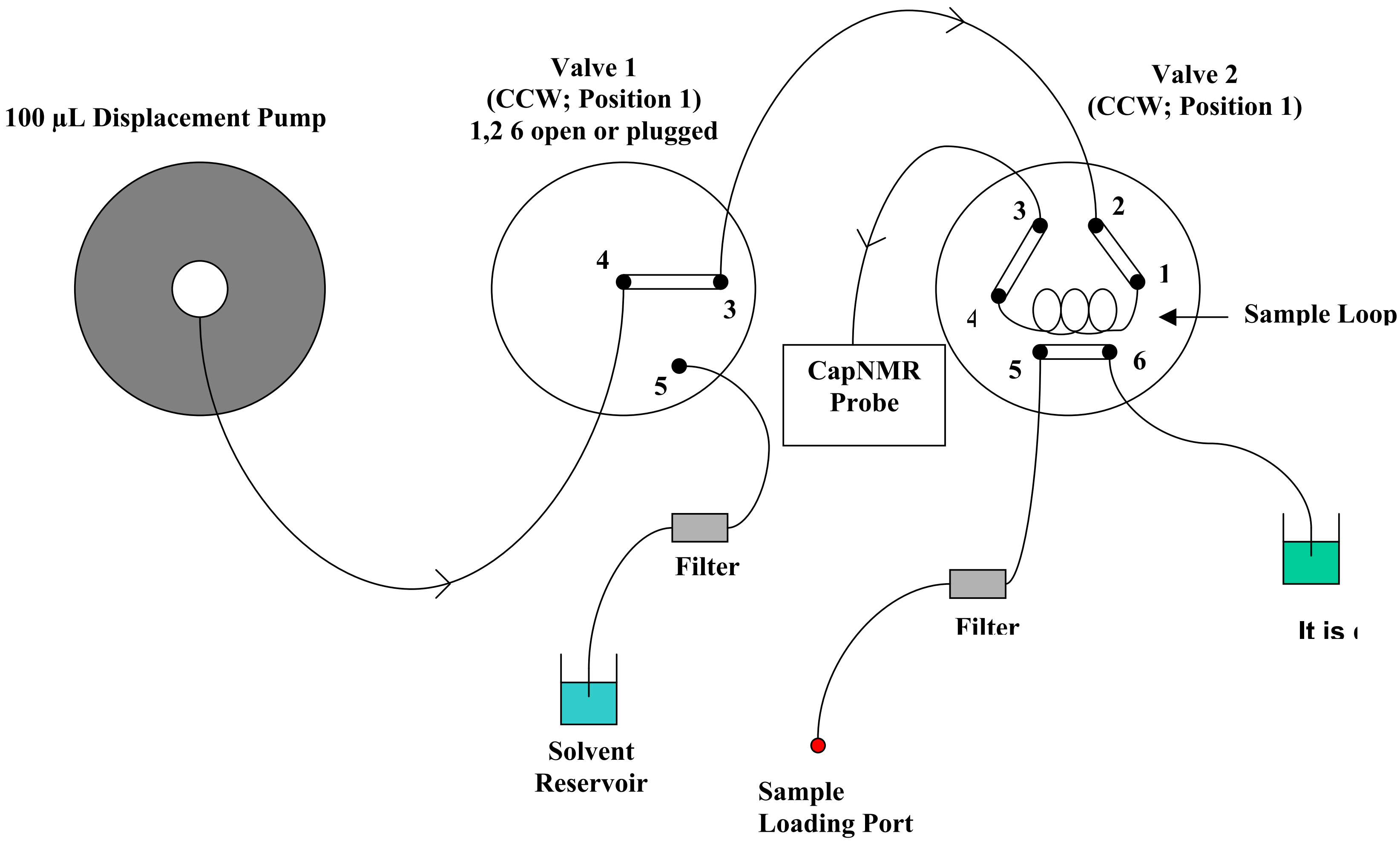
HTSL Capillary Layout

Manual Injection Configuration with FEP

FEP 1/32” tubing, 75-µm i.d. unless otherwise indicated

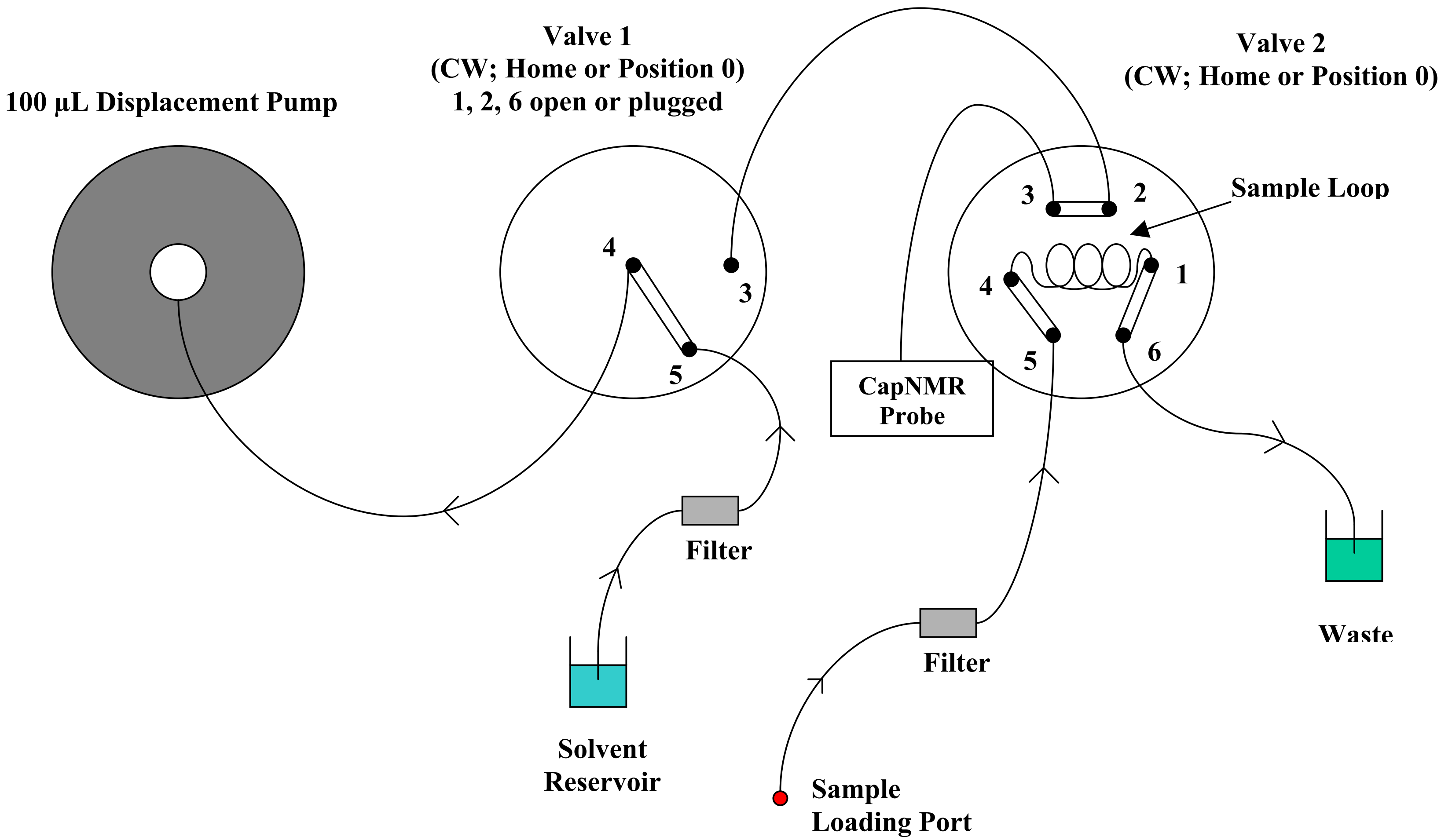


HTSL Fluidic Valve Diagram FEP Version
Status: Deliver



HTSL Fluidic Valve Diagram FEP Version

Status: Fill / Load Loop / Sample Parked / Ready



Parts List Explanation

Here is a guide to using some of the parts for the HTSL-1100.

In the Manual Loading Assembly, to accommodate fused silica users, we recommend Valco bulkhead union #ZBU1XC with a 150 um through-hole disallowing the metal needle tip from contacting the capillary. This union is fitted with the appropriate (large) green sleeves: Upchurch #F-242 for the capillary, and #F-247 for a two-inch, 22s needle. Use the Upchurch black PEEK SealTight ferrule (#F-192) on both ends, as well. Use the Upchurch black PEEK hex nut, #F-287, on both sides of the port.

For connection to the pump, use an Upchurch #U-400 stainless steel hex nut fitted with a #LT-100 PEEK ferrule and compression ring, and a #F-230 orange sleeve.

Capillary i.d. should be 150 μm for the link between the pump and V1, and the link from V1 to the solvent reservoir filter. All other capillary should be 50 μm i.d. All capillary o.d. is 360 μm. It is reasonable to make the loop capillary 150 μm i.d. if desired, but it is probably unnecessary and not optimal especially for partial loop injections.

I recommend no filter in the solvent reservoir itself, but instead, the Upchurch #A-314 Inline Filter in the line between the reservoir and the pump. This way, if the solvent is changed, the filter is self-rinsing, and no bulky filter need be rinsed or dried with precious deuterated solvent. An additional filter is included just downstream of Valve 1 where it connects to Valve 2. Another M-530 is fitted to the sample loading port so each sample loaded into the loop is filtered.

Finally, the recommended priming syringe is the 1 mL Hamilton Teflon Luer Lock Gastight. Hamilton Model #1001TLL, Alltech #81320.

What follows is a list of parts commonly used in the system.

Fluidics Start-Up Kit

Basic Kit (Customer receives all listed parts)

Part[1]	**Minimum**	**Spares**	**Total**
Union, P-779-01	4	1	5
Nut & ferrule, for union, F-331N	8	2	10
Sleeve, F-242	11	4	15
Sleeve, F-247	1	1	2
Filter, M-135	3	1	4
Filter, A-314	1	1	1
Filter end fittings M-130 (for M-135)	1	1	2
Filter disks A-100 (for A-314)	1	1	2
Sleeve, F-185	6	4	10
Nut & ferrule, black PEEK, F-287	4	1	5
Nut, tan PEEK, F-120	2	1	3
FEP tubing[2], 1522	12 in	0	12 in
Plug, P-550	2	1	3
Valco union[3] (Part #ZBU1XC)	1	0	1
Ferrules[4], V-472-11	6	9	15
Valve clamps black, V-472-10	8	0	8
Valve clamps gold, V-580-10	1	0	1
Luer adapter, P-659	1	1	2
Syringe, 10 µL, Sample, Alltech 80065	1	0	1
Syringe, 1 mL, Priming, Alltech 81320	1	0	1
Wrench, ¼" x 5/16", open-end	1	0	1
Wrench, 3/32", Allen	1	0	1
Wrench, 3/32", Allen straight	1	0	1
Nut extender, P-298	1	0	1
Power cord	1	0	1
DB9 cable with only three pins	1	0	1

Fused Silica Capillary Option

Part	**Minimum**	**Spares**	**Total**
Fused silica capillary cutter, FS-315	1	0	1
Fused silica capillary[5], 75/360; Polymicro Technologies, Phoenix, AZ TSP075375, P#2000019	6 m	none	6 m

PEEK Capillary Option

Part	Minimum	Spares	Total
PEEK capillary cutter, A-350	1	0	1
PEEK capillary[5], 1570[6]	6 m	none	6 m
Nut & ferrule, black PEEK, F-287	1	0	1

[1]All parts from Upchurch unless indicated otherwise
[2]Comes in a 10-ft section; cut to 12"
[3]Could be included in manual loading port assembly
[4]Includes 6 ferrules already mounted in valves
[5]Continuous section is available
[6]Upchurch part number for the 5-ft section; a 6-m section would have a custom part number

HTSL-1100 Manual Loading Kit – Extra Optional Items

General Supplies:
Capillary, fused silica; 150 µm i.d., 360 µm o.d.; 2 meters (Upchurch #FS-115)
Capillary, fused silica; 75 µm i.d., 360 µm o.d.; (Polymicro TSP075375, P#2000019), 8 meters
Sleeve, small, green, for capillary filter (Upchurch #F-185)
Union, capillary (Upchurch #P-779-01)
Fittings for the P-779-01; nuts & ferrules (Upchurch #F-331N); sleeves (Upchurch #F-242)
Filter (in-line with capillary, Upchurch #M-135). Note: probe comes with its own filter.
Filter; A-314 for solvent pick-up filtration
P-659 PEEK Syringe Luer adapter
Acetone sample; acetone-p_6 diluted 1:1000 in 90/10 in a Parafilm-sealed Waters bottle
Small volume of acetone-p_6 and squirt bottle
D_2O
Priming solvent (90/10 sonicated and blanketed in helium)
Waters Sample Vials and Caps

Glass Vial, double taper with pre-slit screw caps	Waters #186000385
Glass Vial, single taper with pre-slit screw caps	Waters #186000327
Plastic Vial with pre-slit screw caps	Waters #186000850
Extra pre-slit screw caps	Waters #186000385
Extra non-slit screw caps	Waters #186000274

Pump:
Nut, stainless steel hex (Upchurch #U-400), for pump outlet to capillary
Ferrule and compression ring, (Upchurch #LT-100), for pump outlet
Sleeve, orange (Upchurch #F-230), for connecting capillary to the fitting
Plug for priming port, tan PEEK (Upchurch #P-550)
Syringe, Priming; 1 mL (Hamilton Gastight 1001TLL, #81320; Alltech #81320)
Priming fittings (Upchurch #F-120x); use with 1/16" FEP tube
Tube, FEP, 1/16" o.d., 0.030" i.d., for priming with syringe
Wrenches, black plastic, two (Upchurch #P-298), for tightening the priming port plug
Wrench, ¼", for tightening the PEEK nut on the pump outlet
Spare fuse

Valves:
Ferrules (Upchurch V-472-11)
Straight allen wrench (3/32")
Bent allen wrench (3/32")

Manual Loading Assembly:
Manual Loading Assembly, cable attached
Sleeve, large, green (Upchurch F-242), for connecting capillary to a fitting
Sleeve, large, green, (Upchurch F-247), for syringe needle
Loading port (Valco ZBU1XC)
Loading port nuts and ferrules (two of Upchurch F-287)
Loading port sleeves (one of F-242 for capillary; one of F-247 for syringe needle, 22 s gauge)
Two solvent reservoir bottles
Syringe, Sample; 10 µL, 22s blunt needle (Hamilton Gastight 1701RN, #80065; Alltech #80065)
Wrenches: ½" for rear loading port nut, 7/16" for front nut; adjustable small crescent wrench

Cutting Fused Silica Capillary

You should have received a brief demonstration of how to cut fused silica capillary during your installation.

As a review, the sequence is:

- Push in the button on cutter with your thumb and hold it down
- Position capillary within cutting wheel
- Tighten gently the gold wheel which holds the capillary in place
- Release the button
- Rotate the cutting wheel about one-half way around
- Push in the button
- Loosen the gold wheel which holds the capillary
- Slip the capillary out of place
- Run your index finger and thumb lengthwise along the capillary and crack the capillary
- Flick the tip, and check the tip with a magnifying glass

Microfilter M-135 Assembly and Use

If a blockage is suspected, first check the capillary tips mounted inside the microfilter.

Next, check or change the filter end fitting.

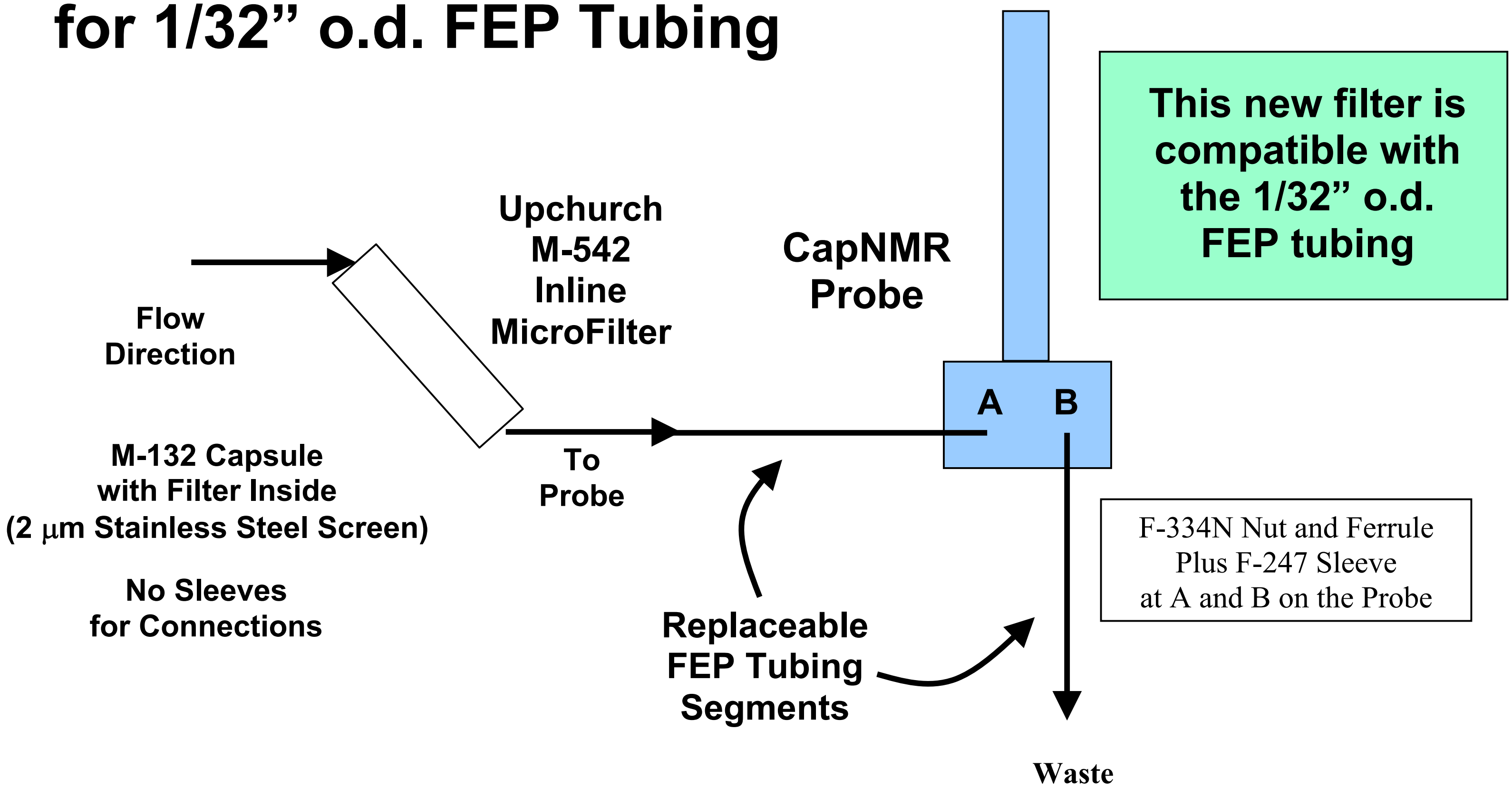
As with any filter, it is a good idea to rinse it upon first use. Also, if a filter is disassembled, the directionality of it needs to be recorded so it is re-mounted in the same direction. If the directionality of a filter is in doubt, replaced it with a new one.

We recommend using the M-135 filter in the direction (shown in the diagram on the next page) such that the fluid enters the end fitting first. This is just a convention, though easy to remember. The standard 360-µm o.d. capillary will require the F-185 tiny green sleeve. In addition, should the M-135 require replacement nuts, they are Upchurch F-125.

The 1-µm stainless steel screen filter replacement end capsule is the M-130.

The 0.5 µm PEEK frit filter replacement end capsule is the M-120. If you want to buy the entire assembly with the PEEK filter mounted in it, order the Upchurch M-520. You may use the PEEK frit anytime you wish, with any solvent except DMSO.

CapNMR™ Microfiltration Scheme for 1/32” o.d. FEP Tubing



It is good practice to rinse a filter before connecting it to the CapNMR probe.

Sample Handling Nomenclature

Loading

- The sample is loaded into the sample loop via the loading port. In the case of the Gilson/HTSL combination, the Gilson needle and syringe constitute the loading device. In the case of the CapLC, the Spark-Holland autosampler needle and syringe constitute the loading device.
- Our Gilson 215 is presently a push-loading device. The CapLC autosampler is a pull-loading device. The Gilson 215 can be changed into a pull-loading device by adding a valve to the Z-arm.

Injection

- The sample is moved from the loop to the detector by an injection device. In the case of the Gilson/HTSL combination, the HTSL is the injection device. In the case of the CapLC, the CapLC pump is the injection device.

Total Sample Volume

- The total volume of sample in a given vial or well.

Pick-Up Volume

- The volume of sample picked up by the needle of an autosampler. This volume must be less than or equal to the Total Sample Volume.

Sample Loop Volume

- The volume of the sample loop alone.

Load Volume

Load volume is the sum of:

- the volume of sample loaded into the sample loop *plus*
- the volume of the plumbing connecting the loop to the loading port *plus*
- any additional sample required to overfill the loop.

Loading Dead Volume

- The volume of plumbing between the loading port and the sample loop.

Injection Volume

- The volume of sample residing in the loop that via pumping is moved toward the NMR flowcell.

Firmware and Software Upgrades

You may have occasion to upgrade the HTSL firmware or software.

Firmware Upgrade

Download the new firmware via the HTSL operating program on the PC (Tools/Download Firmware). Direct the cursor to the correct firmware version (such as 2_41.phf) and press “Open”. Wait till the message box appears saying “Firmware Download Complete” and respond OK. The HTSL will re-initialize; wait for it to finish.

In the PC program, verify via Tools/Instrument Info that the latest firmware is loaded onto the HTSL as desired.

The HTSL is ready for use.

Software Upgrade

Begin with the HTSL off.

On a PC using Windows 2000, NT, or XP, unzip the provided file from Protasis, and move all the unzipped files to your favorite folder on the hard drive of the PC to be used to operate the HTSL.

Install the PC program onto your computer (open the install.exe program).

Close, then re-open the PC software and verify that the latest version is being used (Help/About Protyle). Proceed to File/Setup, then declare the proper COM port (usually COM port 1).

Choose File/Connect, and poise the software to connect to the (still off) HTSL. The status box will indicate that the instrument connected, but not yet seen. If this does not occur, choose File/Setup, and declare a com port. Then, choose File/Connect.

Power up the HTSL and wait for it to complete its initialization routine. (First, the Load and Ready LEDs will blink alternately indicating emptying, then the Load LED alone will blink indicating filling).

An error message appears saying that the current (old) firmware in the HTSL will not work with the current (new) software, and that the firmware should be updated after the HTSL finishes initializing. Respond OK. Another message says that parameter setting and retrieval will be disabled; again, respond OK. After HTSL initialization is complete, the HTSL will be in “Ready” mode with the Ready and Power LEDs steady on. Then, download the new firmware as described above.

Estimating Pressures: Viscosity, Flow Rate, Capillary

To estimate the pressure for a certain flow rate, capillary length, capillary i.d., and fluid viscosity, use the following equation:

$$P = 26 \bullet F \bullet L \bullet \left(\frac{\eta}{1.0}\right)^1 \bullet \left(\frac{50}{d}\right)^4$$

P	**= Pressure in psi**
F	**= Flow in μL/min**
L	**= Length of tubing in meters**
η	**= Viscosity in centipoise (cP)**
d	**= Tubing inner diameter in μm**

In this version of the equation, the rightmost term drops out for 50-μm i.d. capillary. For instance, for a flow rate of 5 μL/min, a viscosity of 1.0 cP (water), and a capillary with an inner diameter of 50 μm, the pressure exerted through a 1-m capillary length is 130 psi. However, a change of capillary i.d. to 75 μm decreases the pressure by a factor of 5.

We can convert the above equation so the final term drops out for 75-um i.d. capillary:

$$P = 5.2 \bullet F \bullet L \bullet \left(\frac{\eta}{1.0}\right)^1 \bullet \left(\frac{75}{d}\right)^4$$

As an example, for a flow rate of 30 uL/min, a viscosity of 1.0 cP, and a capillary i.d. of 75 μm, the exerted pressure through 4 meters of tubing is estimated as 624 psi.

The HTSL-1100 has a maximum pressure limit of about 2500 psi (173 bar).

Note that the pressure applied to achieve a given flow rate is directly proportional to the fluid viscosity.

Fluid	Viscosity (cP) at 20°C
Acetone	0.32
Acetonitrile	0.37
Carbon tetrachloride	0.97
Chloroform	0.57
Dimethyl sulfoxide (DMSO)	2.24
Ethanol	1.19
Methanol	0.60
Sulfuric acid	19.1
Water	1.005

Capillary Assembly and Performance
- Manual Loading Configuration -

Connection	Capillary	Total Length
P – V1PC	150/360	20 cm
V1P1 – Reservoir	150/360	30 cm(v) FS + 30 cm PEEK (Filter inserted)
V1P2 – V2P2	75/360	30 cm (v) + 30 cm = 60 cm (Filter inserted)
V2P5 – Load Port	75/360	25 cm(v) + 40 cm = 65 cm (3 μL; Filter inserted)
V2P1 – V2P4 (Loop)	75/360	1 of 65 cm; 3 μL loop total volume
V2P3 – Probe	75/360	100 cm
V2P6 – Waste	150/360	75 cm

Length to Cut	Number to Cut
150/360 Capillary	
20 cm	1
30 cm	1
75 cm	1
75/360 Capillary	
30 cm	2
75 cm	1
25 cm	1
40 cm	1
65.0 cm	1 (accurate length; 3.0 μL; loop)
100 cm	1 (for use on-site only; connect to filter attached to V2P3 outlet)

Parts

Filter, M-135; three (plumb two at MRM, and add one on-site for connection to V2P3 outlet to probe)
Filter, A-314; one (plumb at solvent reservoir pickup with 0.030” green PEEK tubing)
Unions, P-779-01; four (plumb two at MRM for loop; add two on-site for connection to V2P3 outlet)
Loading Port, Valco ZBU1XC; one; manual loading device

Fluidic Assembly

Mount capillary in all ports
Install three of four filters
Install two unions for testing loop (but ship loop separately, unattached to HTSL)
Install latest HTSL firmware version
Attach labels to capillaries for Probe, Loader, Solvent, Waste, and all 150-μm i.d. segments

Performance Checks

Set F = D = V = 25
Set pump to priming position
Prime with D_2O
Push fluid with 10 μL syringe via loading port, through loop, and out waste (while Ready or Filling)
Prime HTSL and initialize
Perform a run and confirm that fluid emerges from capillary attached to V2P3 (outlet to probe)

Shipping Protocol

Set F = D = V = 25
Set pump to ready position
Remove fluid from pump head
Disassemble loop
Tape capillaries into place
Ship in original box with modified foam bracket; include kit and installation supplies

Valve Status Chart

CW indicates the clockwise valve position from the front. CCW means counterclockwise.

Condition	**Valve 1**	**Valve 2**
Case 1		
Off	CW	CW
Ready	CW	CW
Filling after Run	CW	CW
Filling After Power-Up	CW	CW
During "Pump Fill"	CW	CW
Completion of "Pump Fill"	CW	CW
Completion of "Empty/Fill"	CW	CW
Case 2		
Delivery during Run	CCW	CCW
During "Pump Empty"	CCW	CCW
Case 3		
Completion of "Pump Empty"	CW	CCW
Case 4		
Emptying After Power-Up	CCW	CW

These were evaluated using Beta 4 control software (v. 0.80.0) and version 2.41 firmware.

Tutorial Checklist

Before your installation is complete, be sure you understand these topics.

This HTSL-1100 Manual

Software operation of HTSL
- Priming
- Cycling to finish prime

Cutting capillary

Using capillary unions

Using a union as an outlet plug

Using filters (see written description and demo filter)

Permanence of capillary on probe

Ferrule clusters and the insides of a microvalve (see Upchurch Catalog); changing capillary

Rinsing probe with acetone and air

Syringe vise

Syringe clamp

Loop changeability

Black button use on manual loading assembly (PC software must be disconnected or closed)

Stopping time and volume calibration procedure

Pressure equation

HTSL 3-pin cable

Firmware download procedure

Editions

All users are welcome to comment on the HTSL Technical Notes by contacting the editor:

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Updates are maintained at www.microNMR.com