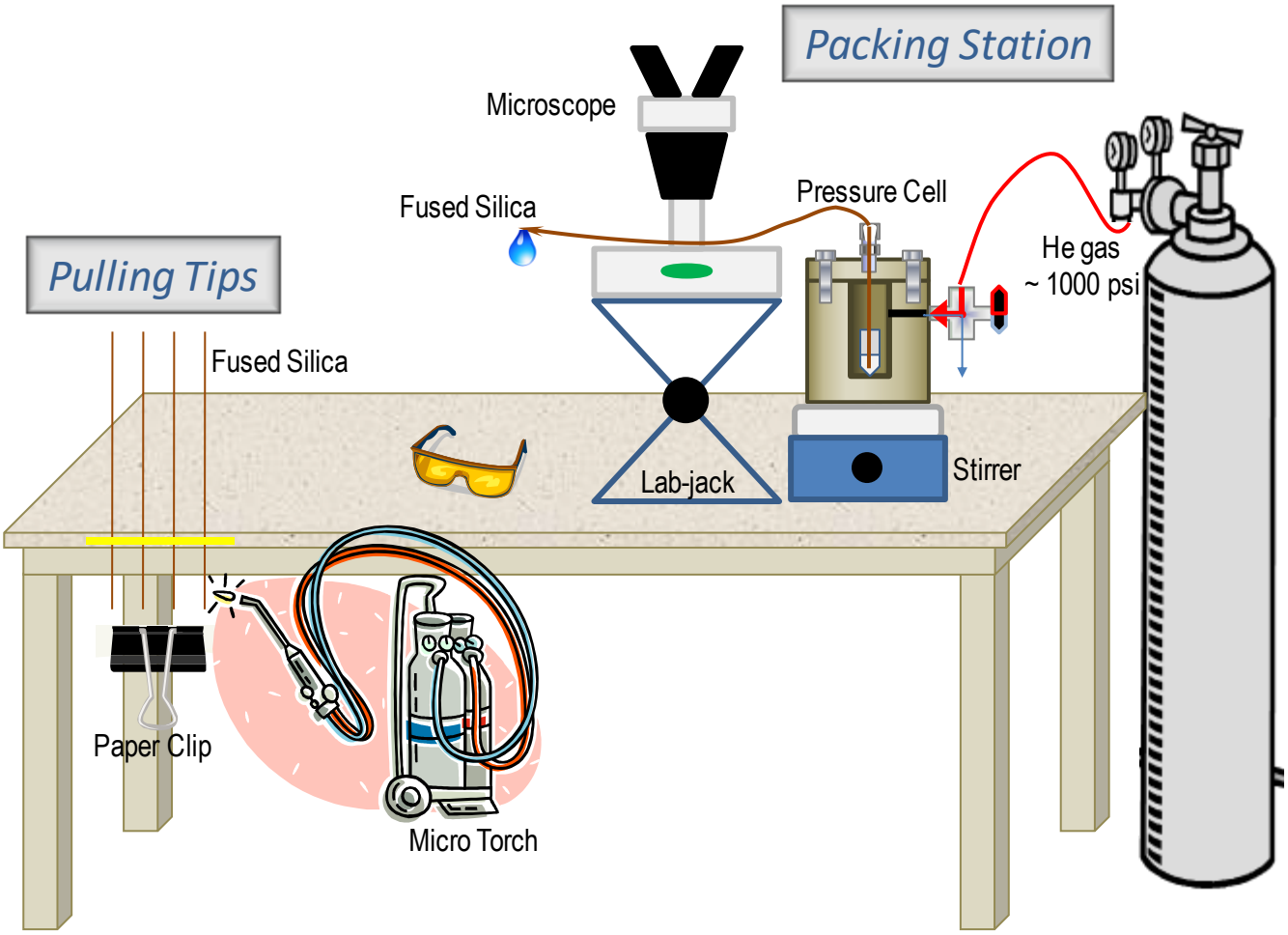
Packing Capillary Columns and Pre-columns (traps)

PPE (personal protective Equipment)

Do not use any of the equipment without the appropriate training. Always wear appropriate PPE when working in the lab, including goggles, lab coats and gloves. PPE is provided, ask if you cannot find the appropriate PPE or if PPE is missing. Always wear goggles when working with fused silica. Review MSDS and SOP's before working with chemicals. Before working with the gas cylinders make sure you complete the EH&S "Compressed Gas Safety" online training (https://www.ehs.washington.edu/training/compressed-gas-safety-online).

Packing Station Overview



Typical setup of a column packing station

The magnetic stirrer and the microscope are optional.

The table at the end of this document list parts needed to setup a packing station as well as some of the consumables. Note most parts can be obtained from multiple vendors, the table only lists examples.

The following sections describe how to pull tapered tips, make a frit and pack columns and traps.

For typical LC-MS setup see the LC-pluming document (https://proteomicsresource.washington.edu/docs/protocols05/LC_plumbing.pdf)

Column/trap End Style

Pulled tip (manual)

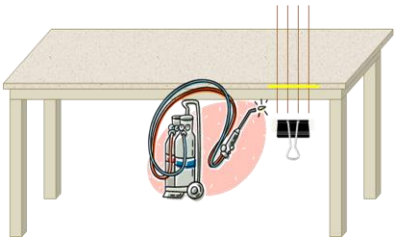
Depending on the resources available to you, you can use a laser tip puller or a micro-torch to pull a thin tip.

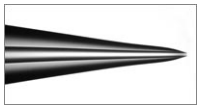
Reagents and Materials (see Table 1 below)

- Fused silica tubing 360 µm OD by 50, 75, 100, 150, 200 µm ID (Polymicro Technologies, see Table 1 below)
- Fused silica cutter
- Micro-torch (e.g. small torch for oxygen/propane (Kingsley North Inc.) or Microflame torch # 14606 (Alltech))
- OR Laser Fiber Puller (Sutter Instruments)

Procedure

Cut desired length of fused silica (5-10 cm longer than final column length) and use tape to attach the fused silica to the side of a table. Attach a large binder clip at the lower end of the fused silica. Use the micro torch to heat fused silica until it melts. The binder clip will act as a weight and pull the tip. Use a fused silica cutter to cut the tip to desired length and desired tip ID.





(Image from New Objective's Website)

SilicaTip™ Size vs. Flow Rate (Recommendation from the NewObjective website)	
Tip Size, ID (µm)	Flow Rate* (nL/min)
5	20-100
8	50-300
10	100-400
15	150-400
30	300-1000

Pulled tip (Laser puller)

Alternatively, you can use a laser puller to pull your tip.

Reagents and Materials (see Table 1 below)

- Fused silica tubing 360 µm OD by 50, 75, 100, 150, 200 µm ID (Polymicro Technologies, see Table 1 below)
- Fused silica cutter
- Lighter, preferable a wind resistant lighter
- Laser Fiber Puller (Sutter Instruments P-2000)

Sutter instruments P-2000

For First time users: Please ask for help when using the laser puller for the first time!

Laser Puller Instructions for pulling ≤375 µm OD fused silica tips

1. Turn Power switch on left side of cabinet ON.
   To assure the most reproducible results you should allow the unit to warm u with the lid down for **15 min** before pulling tips
2. Type Program # and hit <Enter> key. On the UWPR instrument, my favorite is #1.

3. ***Prepare fused silica***: the plastic sheath **MUST** be stripped to expose the quartz glass before loading into the puller. Use the flame of a lighter to burn off about 2 cm of the coating. Wipe CLEAN with a methanol soaked kimwipe.
4. Load the fused silica into the puller:
5. Close lid, be careful not to bread the fused silica
6. Press <Pull>. The glass should separate in a few seconds.
7. Loosen the clamping knobs to remove the fused silica

NOTE: if the fused silica is not aligned properly it will not separate and the red light will stay on. Press <Stop>, open the lid and re-align the fused silica. Close the lid and try again.

To select a different program

1. Hit the <Reset> button, wait for welcome screen to appear
2. Type program # and hit <Enter>

To create a new program

1. ***Be courteous to others and do NOT overwrite someone else's programs!***
2. Select an unused program (see spreadsheet)
3. enter the values as desired.
4. hit <Pull>
5. If you wish to keep your program, indicate so on the spreadsheet by writing down the values of your program

HEAT (laser power output):
Range ~200-350 (if your program and glass require HEAT greater than 350 to get separation, then there is a problem. Ask for Help!
Generally, higher HEAT tends to give longer finer tips

FIL (Filament, scan length):
Range 0-5; This is the scan length of the laser. The manufacturer recommends to use 0 for fused silica. However heating up a longer stretch of fused silica helps generating longer tips. Note extending the scan length may require higher HEAT to get the glass to separate.

Filament #	Scan Length
0	1 mm
1	1.5 mm
2	1.9 mm
3	4.5 mm
4	6.5 mm
5	8 mm

VEL (Velocity, trip point):
Range 15-35; Changing the Velocity will affect the thickness of the glass. Generally if the glass is thinned too much, reduce heat (increments of 10) and increase the velocity (increments of 2), and vice versa.

DEL (Delay)
Range 0-255 (>128 recommended); Controls the timing of the start of the hard PULL relative to the h deactivation of the laser. If the delay is set to 128 the hard pull is initiated at the same time as the deactivation of the laser. If PULL set to 0, the delay allows the glass to cool before the laser activates again, i.e. 128 = no cooling. For fused silica 128 or greater is recommended.

PULL
Range 0-255 (0 recommended); Generally not required for fused silica.

Commercial Frit

Commercial frits are available, e.g. NewObjective's IntegraFrit™ see table 1 below for part numbers

OR make your own frit:

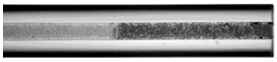
Sintered silica frit

Reagents and Materials (see Table 1 below)

- Fused silica tubing 360 µm OD by 50, 75, 100, 150, 200 µm ID
- LiChrosorb Si60 5µm underivatised silica (e.g. FisherScientific # M93881)
- Fused silica cutter
- Micro-torch (e.g. small torch for oxygen/propane (Kingsley North Inc.) or Microflame torch)

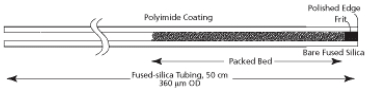
Procedure

1. Cut desired length of fused silica
2. Tamp one end of the fused silica into the Lichrosorb Si60, 30 times.
3. Pass this end quickly through the micro torch flame twice to sinter the frit. It should look similar to this frit.

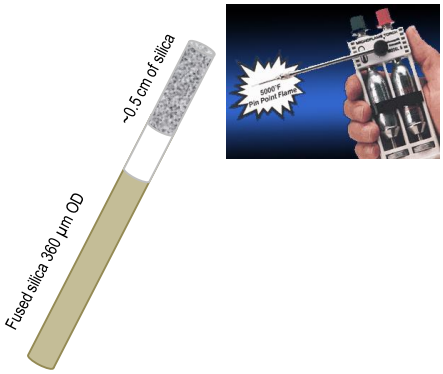


(Image from New Objective's Website)

4. Pack the new frit at 1000 psi and watch to make sure the frit holds



(Image from New Objective's Website)



Note the frits can be reused multiple times.
We use the HPLC pump to remove the packing material. Just reverse the fritted fused silica and start the pump at the necessary flow rate, observe the open end of the fused silica until beads flow out and droplet is clear (all the beads are eluted). Check under a microscope to make sure the fritted fused silica is empty.

Kasil frit

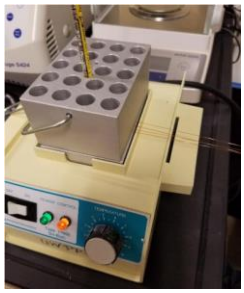
Procedure is based on a method described by Meiring, H.D. etal., J.Sep Sci., 2002, 25, 557-568.

Reagents and Materials (see Table 1)

- Fused silica tubing 360 µm OD by 50, 75, 100, 150, 200 µm ID
- Fused silica cutter
- KASIL 1 Potassium Silicate Solution (29.1%) or Potassium Silicate, Aqueous Soln, 29.8 Deg. Baume, Pfaltz & Bauer No.:P23830-200g (Fisher part # 50-828-816)
- Formamide, 99.5%, for analysis, ACROS Organics (Fisher part # AC205821000)
- Block Heater or Oven 80-90 °C
- Microscope

Procedure

- Cut fused silica tubing lengths to ~25cm (or longer if needed).
- Use a 1.5 ml Eppendorf tube to make up the frit material this has to be made up and used quickly:
- Add 200µL KASIL 1 Potassium Silicate Solution (29.1%) first
- Add 50µL Formamide (you can try 100 ul but it may set too fast) and vortex well for a few seconds.
- Or
- 170µl Potassium Silicate, Aqueous Soln, 29.8 Deg
- Add 30µL Formamide (you can try 100 ul but it may set too fast) and vortex well for a few seconds.
- Spin at 10,000 rpm for 2 min
- Working quickly, place each capillary tube (or as many as you can hold flat between your fingers) into the KASIL/Formamide solution, but not into the pelleted precipitate.
- Capillary action will quickly move the solution up the tube so you only need them in for a brief time. Fill tube with ~1- 2cm of solution.
- Wipe off the outside of the tubing with a kimwipe (all of the bundle can be done at once if it was held flat)
- Inspect under the microscope to see that they are filled to ~1-2 cm to be able to later cut them down to a final length of ~2mm. You want the material to be solid, not broken up as it filled the capillary, though you only care about the portion that will be left.
- Place capillary tubes under a heating block at 90°C. Leave overnight.
  OR
- Curl the bundle into a ring, but do not bend the tubing at the filled end, as this may fragment the frit material and weaken it. Heat in an oven at 80-85 °C, we normally do about 6 hours this may be overkill.
- Remove tubes from heating block or oven and check each for consistency under a microscope.
- Trim with a tubing cutter before use. About 2 mm gives a good amount of backpressure to the column.
- Using the bomb, pack frit with your packing of choice.



Column Packing

Reagents and Materials (see Table 1)

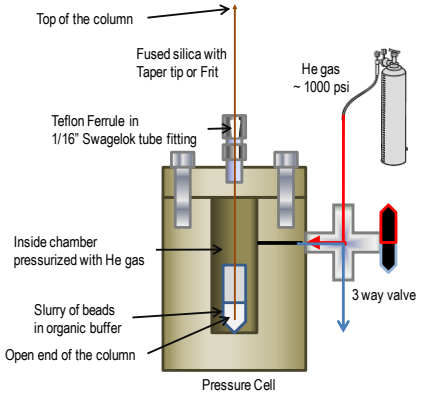
- 80% ethanol (50-100% ethanol, methanol, isopropanol or acetonitrile have successfully been used)
- Packing material: derivatized beads, e.g. Reprosil-Pur 120 C18-AQ, 5 um (ESI source solutions)
- Fused silica tubing 360 µm OD by 50, 75, 100, 150, 200 µm ID (Polymicro Technologies)
- Column end fittings, frits, and tubing sleeves (optional)
- 1.5ml Eppendorf vials
- Small stir bar
- Microscope and/or light source

Pressure Cells

Brechbuehler

Nanobaume

Next Advance



Procedure

1. Cut a section of silica tubing to desired length and pull a tapered tip or make a frit as described above. The other end of the column will remain "open".
2. To make slurry poor (don't use a spatula to avoid contamination of the stock) a little bit of the packing material (~50 µl dry volume) into an Eppendorf vial and add about 1ml of 80% ethanol. Vortex to mix thoroughly, add a small stir bar in the bottom of the vial. Then cut off the cap with a razor blade and place the vial into the pressure cell chamber. Turn stir plate below cell on. (Note the use of a stirrer is optional)
3. Put the top of the pressure cell in place and tighten the bolts.
4. Thread the "open" end of the capillary into the top of the pressure cell – through the nut, Teflon ferrule and top of the cell. The end of the capillary inside the pressure cell should sit a few millimeters above the bottom of the vial. This is best achieved by pushing the tubing down until you feel it hit the bottom, and then backing it off a bit. When you have it to the correct height, tighten the nut on top of the cell tightly with your fingers. Do not use a wrench for this; it will destroy the Teflon ferrule.
5. Set the high pressure regulator on the He gas tank to about 1000 psi.
6. Slowly increase the pressure in the cell by turning the valve 180º clockwise. You should see a liquid droplet forming at the top of the column.
7. Use a microscope or a light source to observe the beads packing and note the flow of the liquid out the top of the fused silica. The packing process may take 20 minutes-1hour, or longer depending on the inner diameter of the tubing being packed and the length of the final column.
8. Once the desired column length is packed slowly release the pressure from the pressure cell by turning the valve 180º counter clockwise. Remove the column.
9. Connect the column to your LC system and equilibrate with the appropriate buffer. Note the pressure cell can be used to equilibrate the column as well, simply replace the slurry with the appropriate buffer and repeat the steps above.
10. Once the column is equilibrated remove the column and cut it to the exact desired length. Note when using a trap column it is best to avoid any dead volume at the end of the chromatographic column by cutting through the packed part towards the end of the column.

Trouble shooting

- **Column is not packing, but liquid droplet is forming at the top of the column (most common case)**
  Gently tap the column until beads flow again (may take a minute or so)
  Packing material could be settling: use stir bar to mix slurry or release pressure and vortex slurry again.
  Open end of capillary is clogged, release pressure and check (cut off the end if necessary).
- **No flow is coming out the top during packing**
  Capillary is probably not far enough into the vial, or is clogged. Release pressure and check.
- **slurry is sliding down capillary when pressure is released**
  This is often observed when the pressure of the cell or from the HPLC is released or if the column was not equilibrated long enough.

Reagents and Materials

Table 1: Reagents and Materials

Part	Description	Vendor	part #
Torch	Torch Oxygen/Propane Kit, includes #4 tip, 6' hoses and regulators for disposable propane and oxygen	Kingsley North Inc.	6-1386
	Tip: #4 Replacement Tip (my favorite tip size)	Kingsley North Inc.	6-1391
	Bernzomatic 1.1 Cubic Foot Oxygen Cyli (189-OX9)	Amazon	189-OX9
	Propane Burner Replacement Cylinder	ThermoFisher	S41897B
	MicroFlame No.4200 - Standard Torch Kit 5000 °F(hard to find)	Azure Moon Trading Corp	No.4200
	Cole Parmer Micro Torch Kit 2500 °F	ThermoFisher	NC9764498
Laser Puller	P2000 Fiber Puller	Sutter Instruments	P-2000/F
Packing stations	Homemade pressure cell click on pressure cell on our tools page https://proteomicsresource.washington.edu/protocols05/pressurecell.php		
	Pressure Cell Kit, includes the cell, fittings, manual, tool kit and ferrules (5 x 0.4mm)	Brechbuehler Inc.	1 1000 100
	Nanobaume™ High Pressure Capillary Packing Assembly, includes the cell, fittings	Western Analytical Products	SP-400
	NextAdvance Pressure Injection Cell is rated for 2500 psi. Includes a three-way valve, hex wrench, 10 Ferrules for typical (~360 um OD) capillaries, a frit kit, and operator manual.	NextAdvance	PC77 PC77-MAG
Accessories	Ferrules (replacement) 100% PTFE, 1/16 in. to 0.4mm	ThermoFisher	NC9656755
	Regulator 1-Stage High Pressure 3000 PSI Brass, 0-2000 Del Range, CGA-580	Airgas	Y11N115H580
	Stainless Steel Pipe Fitting, Hex Coupling, 1/4 in. Female NPT	Swagelok	SS-4-HCG
	SS Swagelok Tube Fitting, Male Connector, 1/8 in. Tube OD x 1/4 in. Male NPT	Swagelok	SS-200-1-4
	Alltech* Standard Stainless-Steel Tubing, 316,1/8X085,50FT	ThermoFisher	AT3010
	Fisher Scientific* Stereomaster* Microscopes	ThermoFisher	12-562-12
	Fisherbrand* Lab-Jack*	ThermoFisher	14-673-52
	Barnstead/Thermolyne* Cimarec* Digital Stirrers	ThermoFisher	11-675-910
	Fisherbrand* Spinbar* Magnetic Micro Stirring Bars	ThermoFisher	14-513-64
Scribe	Chromatography Research Supplies INC Column Scribe 10/PK	ThermoFisher	NC9325879
Frit/Trap	Silica underivatized (if a frit is needed) EMD CHEMICALS LICRSB SI60 LCH 5UM 10GM	ThermoFisher	M93881
	Self-Pack IntegraFrit Columns	New Objective	IF360-100-50-N-5
	KASIL 1 Potassium Silicate Solution (29.1%)	PQ Corporation PO Box Valley Forge PA 19482	call for a sample
	Potassium Silicate, Aqueous Soln, 29.8 Deg. Baume, Pfaltz & Bauer No.:P23830-200g	Fisher	50-828-816
	Formamide - BioUltra, for molecular biology, ≥99.5% (T)	Sigma-Aldrich	47671-250ML-F
	Formamide, 99.5%, for analysis, ACROS Organics	Fisher	AC205821000
	Block Heater (or Oven 80-90 °C)	VWR	12621-108
	BOEKEL Economy Lab Ovens	BioExpress	O-2120-07
Fused Silica	TSP075375 fused silica, 75 µm ID x 360 µOD	Polymicro Technologies	2000019
	TSP100375 fused silica, 100 µm ID x 360 µOD	Polymicro Technologies	2000023
	TSP050375 fused silica, 50 µm ID x 360 µOD	Polymicro Technologies	2000017
Packing material	Dr Maisch Reprosil-Pur 120 C18-AQ, 5 um (1 gm)	ESI Source Solutions	r15.aq.0001
	Dr Maisch Reprosil-Pur 120 C18-AQ 3 um (1 gm)	ESI Source Solutions	r13.aq.0001
	Column Packing Material Jupiter 4u Proteo 90Å, 1 gr	Phenomenex	04A-4396
	Column Packing Material Jupiter 5 u C18 300Å Bulk Packing, 1 gr	Phenomenex	04A-4053

	Other vendors of bulk packing material include: Phenomenex, Alltech, Vydac, Restek, ZirChrom… see Table 2 below

Table 2: Bulk Column Packing Media

Vendor	Part #	Description	Chemistry	Pore Size	Particle size
Phenomenex	04A-4396	Jupiter 4u Proteo 90A, 1 gr	C12	90A	4u
Fisher	AT16082	Adsorbosphere C18 10 U 80A 10g	C18	80A	10u
Michrom	PM3/91100/00	Halo 2.7um 90A C18	C18	90A	2.7u
Michrom	PM3/66100/00	Magic C18 100A 3u PM3/66100/00	C18	100A	3u
Michrom	PM5/66100/00	Magic C18 100A 5u PM5/66100/00	C18	100A	5u
Fisher	06-716-307	Restek Ultra 5microm Bulk Packing Material Ultra C18 Bulk Packing, pore size: 100Å, carbon load: 20%, endcap: fully endcapped, pH range: 2.5 to 7.5	C18	100A	5u
Fisher	06-717-238	Pinnacle II C18 Bulk Packing, 5 microm,110Å , carbon load: 13%, endcap: fully endcapped , pH range: 2.5 to 10 , min order 5gr	C18	110A	5u
Michrom	PM5/66200/00	Magic C18 200A 5u PM5/66200/00	C18	200A	5u
Phenomenex	04A-4053	Jupiter 5 u C18 300A Bulk Packing, 1 gr	C18	300A	5u
Fisher	AT16088	Adsorbosphere C18, 10g 5u	C18		5u
Michrom	PM3/61100/00	Magic C18AQ 100A 3u PM3/61100/00	C18AQ	100A	3u
Michrom	PM3/61200/00	Magic C18AQ 200A 3u PM3/61200/00	C18AQ	200A	3u
Michrom	PM5/61100/00	Magic C18AQ 100A 5u PM5/61100/00	C18AQ	100A	5u
Michrom	PM5/61200/00	Magic C18AQ 200A 5u PM5/61200/00	C18AQ	200A	5u
Michrom	PM3/67100/00	Magic C30 100A 3u PM3/67100/00	C30	100A	3u
Michrom	PM5/67100/00	Magic C30 100A 5u PM5/67100/00	C30	100A	5u
Michrom	PM3/64100/00	Magic C4 100A 3u PM3/64100/00	C4	100A	3u
Michrom	PM5/64100/00	Magic C4 100A 5u PM5/64100/00	C4	100A	5u
Michrom	PM3/90100/00	Halo 2.7um 90A C8	C8	90A	2.7u
Michrom	PM3/60100/00	Magic C8 100A 3u PM3/60100/00	C8	100A	3u
Michrom	PM3/60200/00	Magic C8 200A 3u PM3/60200/00	C8	200A	3u
Michrom	PM5/60100/00	Magic C8 100A 5u PM5/60100/00	C8	100A	5u
Michrom	PM5/60200/00	Magic C8 200A 5u PM5/60200/00	C8	200A	5u
Fisher	06-717-157	Pinnacle II C8 Bulk Packing, 5 microm	C8		5u
Michrom	PM5/62100/00	Magic Cyano 100A 5u PM5/62100/00	Cyano	100A	5u
Michrom	PM5/63100/00	Magic Phenyl 100A 5u PM5/63100/00	Phenyl	100A	5u
Fisher	M93881	EMD CHEMICALS LICRSB SI60 LCH 5UM 10GM	si	60A	5u