

## Procedure for building your own trap column

Before starting to pack your own column, please note that all items (peek tubings, ferrules, etc) are stored in their own plastic bag with their specifications on the bag, date they arrived, used/new status, etc; which we use for ordering. Therefore, please remember to put everything back in their bags, otherwise it would be confusing to find the correct specifications again for ordering.

**If you use the last of any of the items, then its your responsibility to make sure new items are ordered.**

### Tools:

- 250 µl syringe
- Injector tool
- 2 spanners
- gloves
- A working station with proper lighting

### Materials needed:

- Reverse phase beads
  - R1 POROS beads are designed for very hydrophobic proteins (large proteins aprox. > 30-40 kDa).
  - R2 POROS beads are designed for less hydrophobic proteins (small proteins)
- Screens (Microlab part no. 2SR1-10 (10pack))
- Tefzel column (transparent tubing) – Can be reused up to 3 times
- PEEK tubing (Please notice that we have two types of PEEK tubing:
  1. Peek tubing, ID (inner diameter) 0.005'' (=0.125 mm), OD (Outer diameter) 1/16'' (=1.58mm). This is in neutral color in Waters, but in Red color in Upchurch (IDEX – part no: 1535, 5 ft (1.5m)).
  2. Peek tubing, ID (inner diameter) 0.010'' (=0.25 mm), OD (Outer diameter) 1/16'' (=1.58mm). This is in neutral color in Waters, but in Blue color in Upchurch (IDEX).
    - a. **The PEEK tubing will be distinguishable through labelling (A, B and Standard)**
- 1/16'' Ferrules (Part no. U401X)
- fittings for attaching to the ASM/BSM
- Methanol (MeOH) / MQ-Water (MQ) 1:1 v/v solution: **USE THE BOTTLE SPECIFIED IN Figure 8B**
  - If empty, be sure **ONLY** to use LC-MS grade glass ware when preparing new solution. Available in fumehood 618.

If all the parts and materials are clean then you can start this procedure.

Column naming.

There are 4 spare columns available at all times (2 R1 and 2 R2). These have been named column 1 through 4. 1&2 contain R1 beads – 3&4 contain R2 beads. **These MAY ONLY be packed by the column colonel (RJ).**

All personal columns have to be packed by yourself and be marked with initials and bead type and column number. Starting from column 5 (figure 1). This insures that your column IS yours.



Figure 1: RJ personal column (No. 9) bead type R2

Column overview		
Name	Column number	Bead type
Standard	1	R1
Standard	2	R1
Standard	3	R2
Standard	4	R2
Nanna (NB)	5	R1
Sidse (SBL)	6	R1
Zeinab (ZEN)	7	R2
Patrick (PSM)	8	R1
Rasmus (RJ)	9	R2
Zeinab (ZEN)	10	R1

Column preparation - You only need to pack only from one side.

1. Clean the metal column with MeOH: MQ 1:1
2. Cut the tefzel column to an approximate length of 2 mm. The column **must** be flat along the edges so the screens and subsequently the PEEK tubing can sit in alignment avoid any dead volume. Should you experience a dead volume, repacking with a slightly longer Tefzel column (2.5-3 mm) could be tried.

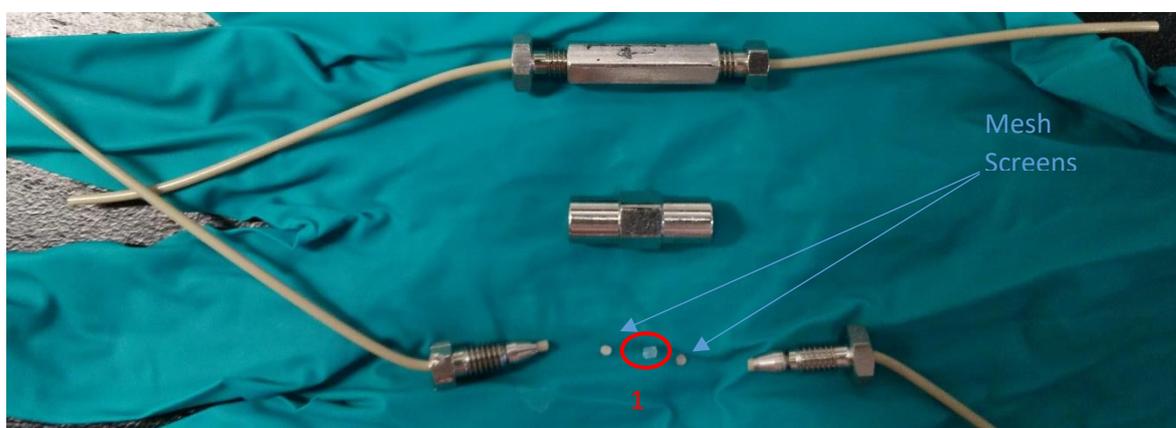


Figure 2. Picture of assembled (upper) and disassembled (lower) reversed-phase trap column. The system consists of a two metal mesh screens with tefzel column inbetween. (1) Tefzel column

3. The steel column is **NOT** allowed to have a narrowing in the middle (Figure 3A). If it does, the tefzel column will **NOT** fit properly resulting in excess dead volume (Figure 3B). If you spot such a column. Please hand it in.

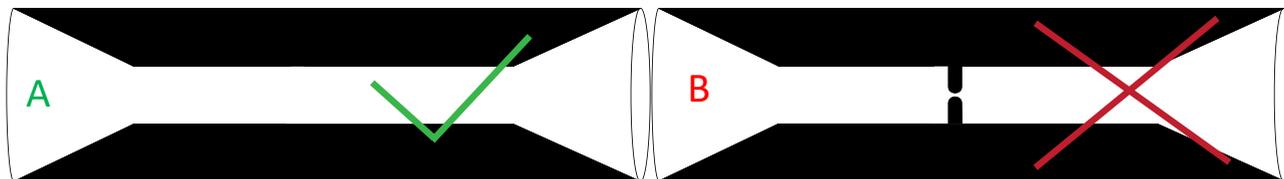


Figure 3

Take the standard tubing and screw it onto union of the cleaned metal column (Figure 4)

- a. Add a screen and stamp it in place using a piece of PEEK tubing (figure 7).
- b. Add the tefzel column on top of the screen and stamp into place using PEEK tubing (Figure 7)

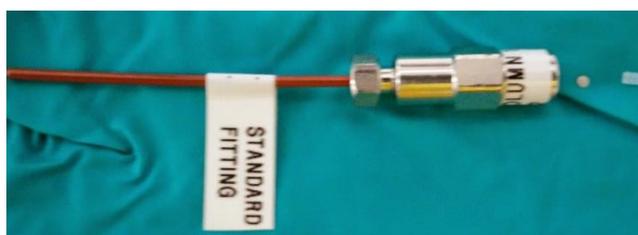


Figure 4

4. Add the packing adaptor to the top of the metal column. Make sure you push down the tip of the packing adaptor down while screwing (Figure 3A + B). This will push the plastic fitting fit right onto the tefzel column, ensuring a snug fit. When all the way in, the plastic tip will stick out like shown on (Figure 3C)

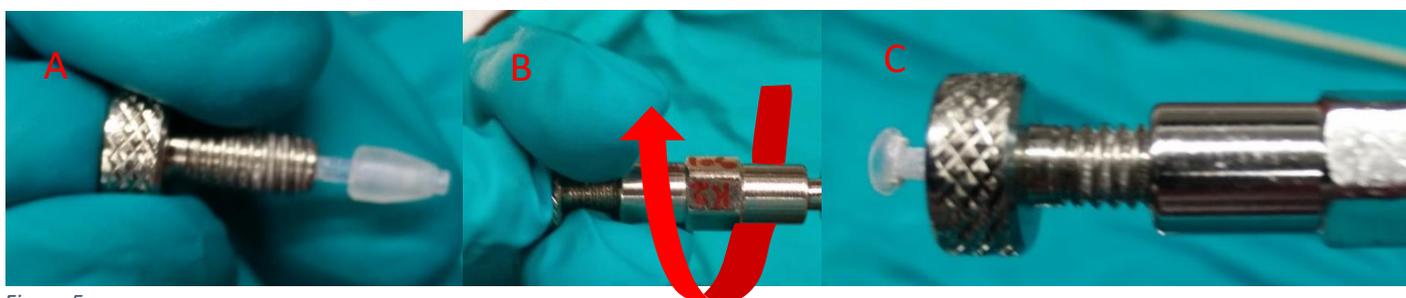


Figure 5

5. Before adding the packing material through the packing adaptor it is a good idea to dilute the bead slurry with 1:1 MeOH/MQ v/v

- a. e.g. add about 25  $\mu$ l of packing material and 500  $\mu$ l of the MeOH/MQ solution, per column your packing
- b. Use the dedicated column packing syringe.
6. Flush with the MeOH/ MQ solution to set the beads in place.
7. After flushing, take off the packing adaptor check to see that it is packed properly (figure 6).
  - a. If the column is as shown in figure 7, then add another screen on top. Make sure the screen is sitting correctly over cut tubing filled with resin. This is done by “stamping” the screen onto the column by reversing the standard tube fitting (figure7).



Figure 6: Notice the neat white circle. This indicates the tefzel column has been packed with beads

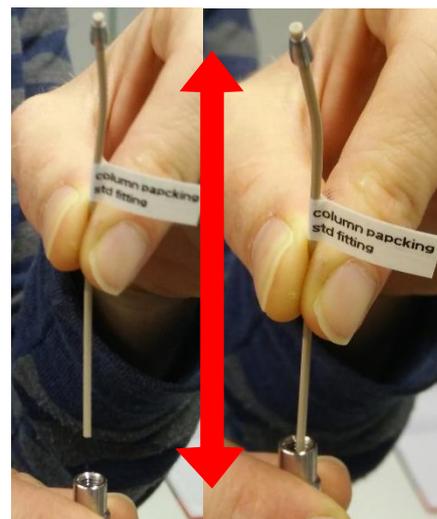


Figure 7. Stamp the screen in place with the other end of PEEK tubing

- b. If you have excess resin material carefully resuspend the excess material with MeOH/MQ and carefully extract the suspended material using the syringe in circular movements. When done perform. **Perform step 6 again to reduce dead volume.**
8. **Reuse ferrule with PEEK tubing A.** Tighten it carefully.
9. Remove PEEK tubing Standard
  - a. If you used a new tefzel column, cut the ferrule of PEEK tubing B and go to step 10c.
  - b. If not, reuse PEEK tubing B
  - c. Add threads, then the ferrule. Push the PEEK tubing all the way into the column, until the tefzel tubing hits PEEK tubing A (figure 8). **Before the ferrule is tightened to the tubing. Make sure the tubing is pushing against the screen.** This will reduce the dead volume in the system and prevent the column from leaking.



Figure 8

- d. During tightening, be careful not to tighten too hard. 1/2-3/4 spanner turn should suffice (figure 9). Otherwise, the inner diameter of the tubing will be too small, subsequently preventing flow.



*Figure 9. The red arrows indicate the amount of tightening that should be sufficient for fitting the ferrule without blocking the PEEK-tubing*

- e. Check the ferrule fitting by unscrewing the thread and test the ferrule:PEEK tubing attachment. If the ferrule is not yet attached tighten further.
10. Install onto the Rheodyne valve and flush the column to prepare it for injection
    - a. It is important to flush the excess packing material away in the system before starting your run so the trap column does not get clogged.
      - i. Flush 1mL MeOH:MQ 1:1
        - I. **USE DESALTING SYRINGE!**
      - ii. Reverse the column and repeat
    - b. After flushing you prepare the column by setting the valve in “inject mode”
      - i. Buffer A: Buffer B - 50:50 for 10 minutes at 40 $\mu$ L/min, followed by
      - ii. Buffer A: Buffer B - 100:0 for 10 minutes at 40 $\mu$ L/min

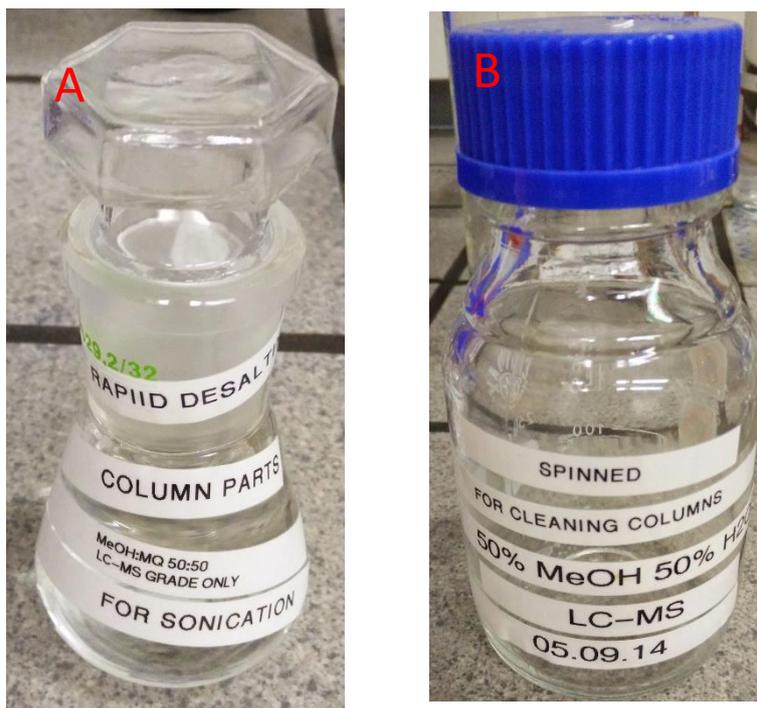


Figure 10: a) Conic flask for sonication of column parts b) cleaned MeOH:MQ 1:1 v/v

Note: **Reuse the tefzel column (up to 3 times) and the screens** They are cleaned with by lowering them into 1:1 v/v MeOH/MQ solution followed by a 10-15 minute sonication **only use the conic flask specified in Figure 10A**. New ones are pricey, so be gentle.

### **Protocol Log:**

Date	Version	Comments/Changes	Responsible
2014-12-24	1.0	New Protocol	RJ
2015-01-06	1.1	Note on peek tubings	ZEN
2015-01-14	1.2.2	Reworked phrasing and changed workflow	RJ
2015-01-18	1.3	Added column numbering	RJ
2015-01-27	1.4	Added Column no. 10 and spare parts numbers	RJ
2019-12-10	1.5	Changed suggested Tefzel column length from 3 mm to 2 mm	AR