

MyCapLC™ Kit Packing Instructions

The following guide provides a starting point for the assembly and packing of capillary columns using the MyCapLC™ Kit. The packing instructions are tailored for the provided C18 stationary phase and are indicative only. Packing conditions may vary with different types and brands of stationary phase.

Kit Contents

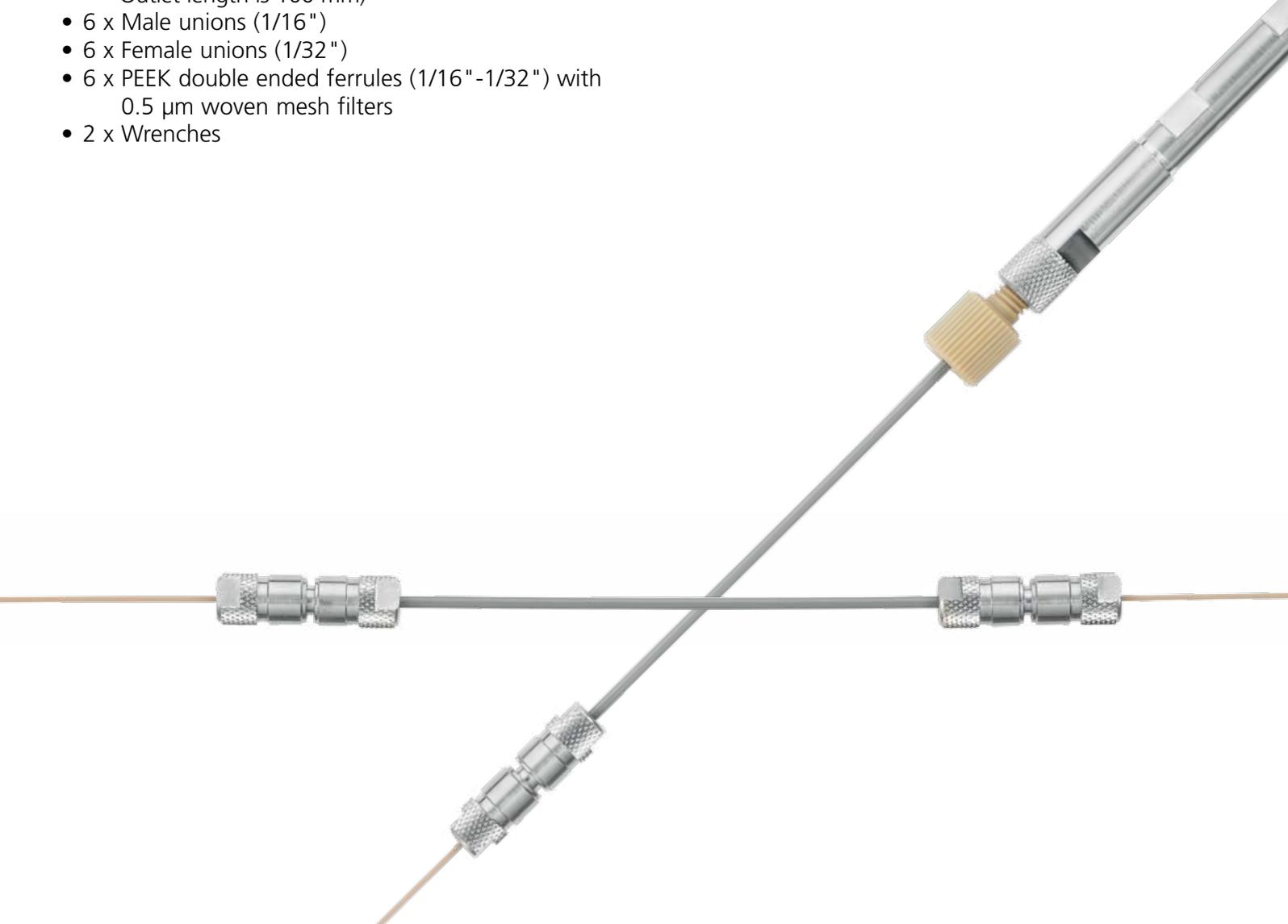
(suitable to pack three columns)

- 0.1 g C18 3 μm 200 Å
- 1 x Slurry reservoir (50 mm x 2.1 mm ID column tubing)
- 2 x End fittings with inserted PEEK sealing rings
- 1 x PEEK Fingertight fitting
- 3 x Column bodies (1/16" OD PEEKsil®, lengths from 50 mm - 150 mm and IDs from 150 μm - 530 μm)
- 6 x Connection capillaries (1/32" and 0.36 mm OD options available in 50 μm ID. Inlet length is 250 mm. Outlet length is 100 mm)
- 6 x Male unions (1/16")
- 6 x Female unions (1/32")
- 6 x PEEK double ended ferrules (1/16"-1/32") with 0.5 μm woven mesh filters
- 2 x Wrenches

Other Items Required

You will also need these items in your laboratory:

- 1 x Large bore syringe and needle, or pasteur pipette
- 1 x HPLC pump
- 1 x Waste container
- Cyclohexanol and chloroform as your slurry solvent
- Acetonitrile in water as your packing solvent



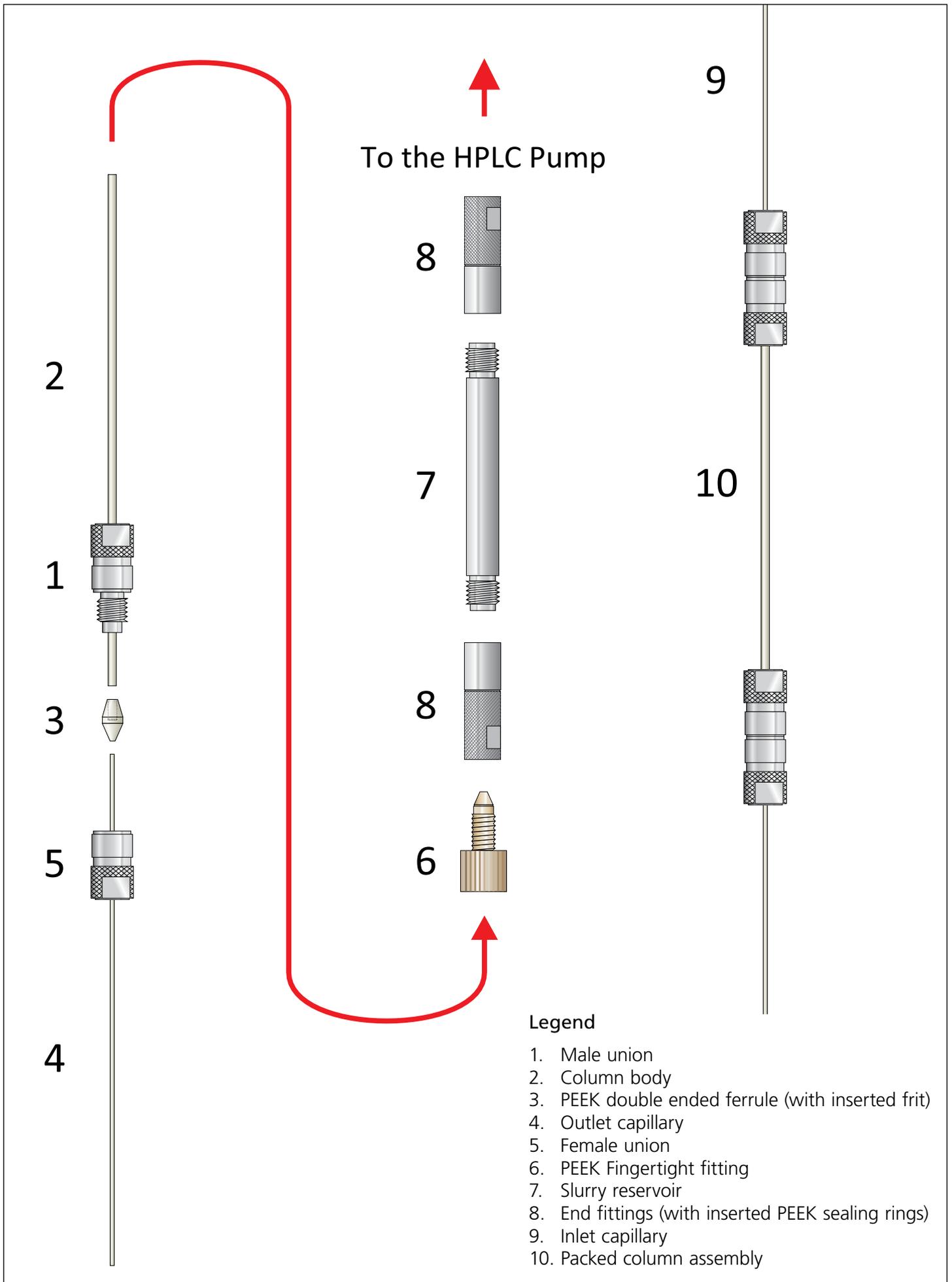


Figure 1. Assembly of column body, outlet capillary and slurry reservoir for packing; then assembly of packed column with inlet capillary.

Instructions

A. Assembling Column Body, Outlet Capillary and Slurry Reservoir for Packing (Figure 1)

1. Slide a male union (1) onto a 1/16" PEEKsil column body (2) with the thread of the male union facing towards the near end of the column.
2. After checking that the frit is still in place within the double ended ferrule (3), insert the end of the column into the 1/16" opening of the ferrule.
3. Holding the double ended ferrule (3), gently push the male union (1) up the shaft of the column body (2) until it meets the double ended ferrule.
4. Place the column body on the bench while preparing the outlet capillary (4).
5. Slide a female union (5) onto the PEEKsil outlet capillary (100 mm in length) (4) with the thread of the female union facing towards the end of the capillary that will be connected to the male union (1).
6. Insert the end of the PEEKsil outlet capillary (4) into the outlet opening of the double ended ferrule (3) at the end of the column body (2).
7. Gently push the female union (5) up the shaft of the outlet capillary (4) until it engages with the male union (1). Turn the union to engage the threads.
8. Gently push the column body (2) and outlet capillary (4) towards each other, to ensure that each capillary end is against the stop inside the double ended ferrule (3); then tighten the male union (1) and female union (5) until they are finger tight.

	CAUTION
	If excessive force on either of the capillary tubes is used, or if the 1/16" column body is not holding the frit in place, the capillary can damage the frit, rendering the column inoperable.

9. Using the wrenches, tighten the union connection a further 90 ° (quarter turn).

	CAUTION
	Be careful when using the wrenches. Do not over tighten – further tightening will cause the capillaries to break.

10. Attach the column assembly, as detailed above, to the slurry reservoir (7) using a PEEK Fingertight fitting (6) threaded into the end fitting (8).
11. If not already done, tighten the end fitting (8) onto the bottom end of the slurry reservoir (7), using the wrenches, approximately a further 90 ° (quarter turn).

B. Preparation of Slurry and Filling the Slurry Reservoir

12. Make a slurry with your chosen stationary phase, ensuring you have the amount of silica required for the column size you will pack (see Table 1).

Column Length		50 mm	100 mm	150 mm
Column ID	150 µm	0.6 mg	1.2 mg	1.8 mg
	300 µm	2.3 mg	4.6 mg	6.9 mg
	530 µm	7 mg	14 mg	21 mg

Table 1. Minimum amount of silica needed to pack capillary columns.

The silica should be suspended in 175 µL of a mixture of 10 % cyclohexanol and 90 % chloroform. If cyclohexanol is not available, 100% chloroform is also suitable.

Column Packing Notes

Cyclohexanol/chloroform and chloroform have been shown to be successful for packing C18 packing material supplied with this kit. Other resins may need different solvents for optimal packing.

13. Open the top end of the slurry reservoir (7).
14. Use a pasteur pipette or a syringe with a large bore needle to introduce the slurry into the slurry reservoir (7). Slowly withdraw the pipette or syringe tip while the reservoir fills up. Gently tap the side of the reservoir to ensure that all air bubbles have been removed.
15. Close the reservoir with the end fitting (8) and hand tighten until finger tight.
16. Using the wrenches, tighten the end fitting (8) onto the slurry reservoir (7) approximately a further 90 ° (quarter turn).
17. Packing should commence as soon as possible to prevent the particles from settling. From this point on, the packing assembly should be kept in a vertical position until the packing process is complete.

C. Packing the Column

18. Attach the slurry reservoir assembly to the HPLC pump by threading a 10-32 fitting into the slurry reservoir end fitting (8), and let the outlet capillary (4) lead to a waste container.
19. Use 60-80 % acetonitrile in water as your packing solvent. Recommended flow rates are as listed in Table 2.

Column Length		50 mm	100 mm	150 mm
Column ID	150 µm	2.5 µL/min	2.5 µL/min	2.0 µL/min
	300 µm	10 µL/min	10 µL/min	8 µL/min
	530 µm	30 µL/min	30 µL/min	24 µL/min

Table 2. Recommended flow rates for packing capillary columns.

During the packing process the back pressure will rise to 3,000-4,000 psi as the column body is filling up (if only chloroform is used as slurry solvent, the pressure will be lower). Once the slurry solvent has passed through the column body the pressure will drop to about half of its maximum value due to the lower viscosity of the packing solvent.

Continue to pack for one additional hour, then switch the pump off. Packing for a longer duration (e.g. overnight) will not do any harm.

20. After the pump was been switched off, wait for the system pressure to dissipate completely before removing the column body (2) from the slurry reservoir (7).
21. Examine the top of the column body (2) to check that it is fully packed.

D. Assembling Packed Column with Inlet Capillary (Figure 1)

22. Slide a male union (1) onto the packed column assembly (10) with the thread of the male union facing towards the near end of the column.
23. After checking that the frit is still in place within the double ended ferrule (3), insert the end of the column into the 1/16" opening of the ferrule.
24. Holding the double ended ferrule (3), gently push the male union (1) up the shaft of the packed column (10) until it meets the double ended ferrule.
25. Place the packed column on the bench while preparing the inlet capillary (9).
26. Slide a female union (5) onto a PEEKsil inlet capillary (250 mm in length) (9) with the thread of the female union facing towards the end of the capillary that will be connected to the male union (1).
27. Insert the end of the PEEKsil inlet capillary (9) into the inlet opening of the double ended ferrule (3) at the end of the packed column (10).
28. Gently push the female union (5) up the shaft of the inlet capillary (9) until it engages with the male union (1). Turn the union to engage the threads.
29. Gently push the packed column (10) and inlet capillary (9) towards each other, to ensure that each capillary end is against the stop inside the double ended ferrule (3); then tighten the male union (1) and female union (5) until they are finger tight.
30. Using the wrenches, tighten the union connection a further 90° (quarter turn).

Congratulations, you have now completed the packing and assembly of your customized column!