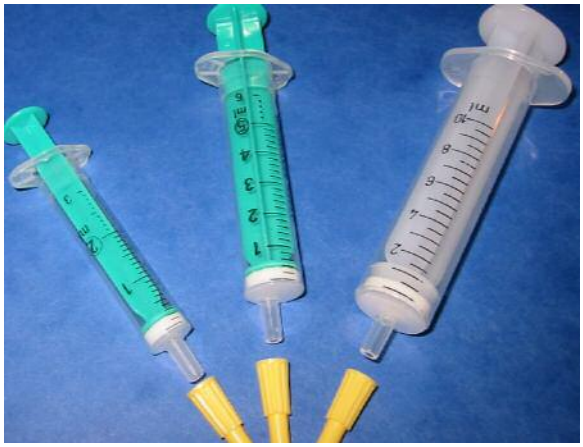
Application note:

Disposable reaction vessels for peptide-resin cleavage



Most peptide synthesizers automate only the assembly of peptides on the solid support, but not the cleavage from the resin. To isolate the desired product, some additional steps are required:

- Drying of the peptide-resin
- Treatment with TFA for cleavage
- Separation of TFA/peptide solution from resin
- Precipitation with ether
- Filtration or centrifugation
- Washing with more ether
- Dissolution and lyophilization

With appropriate tools and procedures this task list becomes much less daunting. We here propose a proven procedure and cheap disposable polypropylene plastic ware for peptide work up.

Equipment needed:

- a filter column with plunger (looks like a plastic syringe fitted with a filter frit
- a polypropylene vial with a screw cap and a sturdy holder for it
- an adapter with Teflon tube to fit the luer tip of the filter column (re-usable)

Safety: Lab coat, gloves and safety glasses must be worn throughout the procedure!

TFA is a very corrosive acid which will lead to severe burns on the skin. Get the safety data sheet from your supplier and observe the instructions. Never work alone in the lab and have emergency aids at hand. Always work in a fume hood. Ether is highly flammable and must be handled with great care.

Reagents and solvents:

Ethanol or dichloromethane (DCM) to wash and dry the resin
Cleavage cocktail: 95% TFA, 2.5% tri-isopropyl silane (TIPS), 2.5% water. Mix well to dissolve
Tert.-butyl methyl ether for precipitation (may be substituted by stabilized diethyl ether)

Procedure:

1. Select an appropriate filter column which will hold at least 3 times the volume of the resin (measured as swollen in DMF):
   50-200 mg resin: 2 ml column
   250-500 mg resin: 5 ml column
   0.5-1 g resin 10 ml column

2. Transfer the resin into the filter column. If required, rinse out your synthesis reaction vessel and pour the slurry into the filter column. Press out excess solvent.

3. Wash the resin in the filter column with a suitable volatile solvent such as dichloromethane (DCM) or ethanol (preferred). Draw up solvent, shake suspension for 10 sec. and press out solvent. Repeat 5 times.

4. Dry the resin by putting the columns (with plunger) in a vacuum system over night.
   Note: If you have weighed the empty column you can conveniently determine the raw yield now.

5. Put an appropriate volume of cleavage cocktail into the polypropylene tube used for further work-up, held in a solid rack. Use approximately 1 ml of TFA for each 100 mg of peptide-resin:
   200 mg resin: 2 ml in 13 ml tube
   1 g resin: 10 ml in 35 ml tube
   Cleavage cocktail: 95% TFA, 2.5% tri-isopropyl silane (TIPS), 2.5% water. Mix well to dissolve.
   Note: a fume hood must be used when handling TFA!

6. Attach a re-usable Luer-adapter with Teflon tube to each filter column. Aspirate the cleavage cocktail into the column. In most cases it will be pressed out again by isobutene gas generated from protection groups cleaved. Carefully press out the gas and aspirate TFA again. Repeat until the TFA stays in the column. Leave the column in the work-up tube, making sure it can not tip over.
   Note: The outlet of the filter column must be in the cleavage tube at all times as TFA may be discharged. The filter column may not be closed during the cleavage procedure! TFA and gas must be pressed out slowly with great care as the Luer adapter could fall off the column tip.

   Lab coat, gloves, safety glasses and fume hood are mandatory!

7. Swirl the resin/TFA mixture occasionally for thorough mixing or dispense and aspirate the TFA. Wait for cleavage time appropriate for the linker and side chain protection groups. 2–4 hours are usually sufficient. Dispense TFA into work up tube and squeeze out the resin after cleavage.

   Note: Copolymer resins such as TentaGel may still hold a considerable volume of TFA. Most of the peptide is in the solution but for better recovery the resin should be washed with a second aliquot of TFA in a second work-up tube.

8. Fill up the cleavage tube (holding no more than 20 % of its volume in TFA) with tert.-butyl methyl ether, which has been cooled on ice. Screw the lid on the tube and mix TFA and ether.
   Note: The mixture will warm up with ether addition. The ether must be cold and its volume must be at least 3x the TFA volume. Add the ether quickly.

9. The peptide should have precipitated now. If not, add a few drops of water and cool the mixture on ice. The precipitated peptide can be filtered off using a fine pore filter, not the filter column! Alternatively, it can be centrifuged at low speed in a spark-proof centrifuge.
10. **Note**: It is illegal to use ether in a normal centrifuge. You must check if your centrifuge is certified as spark-free and use a secondary closed container as well. Please contact your safety administrator before proceeding. The precipitated peptide should be washed with ether two more times.

11. After the last wash the ether is decanted and 1 ml of water added to dissolve the peptide. Hydrophobic sequences require addition of up to 30 % acetonitrile, very acidic peptides a drop of ammonia solution. Freeze the solution, even if it is not dissolved. Freezing the tube at an angle of 45 degrees will increase the surface area for lyophilization.
    Note: The peptide precipitate may not be dried before adding water! The residual ether is easily removed after freezing.

12. Freeze dry the peptide. Weight the yield and dissolve it again for analysis or use in a biological assay.
    Note: HPLC analysis is possible only after complete removal of ether. The quality of the peptide must be checked by mass spectrometry.

13. Dispose of reagents, vials, columns according to the safety regulations applied in our lab.