

### Desalting of gel-extracted peptides with 100 µl C18 tips

These pipet tips with a monolithic block of C18 reversed phase material at the very end, capacity is up to 50 µg peptide. They are used just by drawing solutions up and down without air. For desalting small amounts use 10 µl tip version.

#### Materials:

100 µl C18 tips  
200 µl pipet  
low-bind Eppendorf tubes  
Racks, 15 ml Falcons

#### Solvents:

0.1 % TFA.  
70 % ACN 1 % FA or 70% ACN 0.1% TFA  
100 % ACN

#### Procedure:

1. dry peptide extract from gel extraction in a vacuum concentrator
2. add 100 µl 0.1 %TFA, vortex, sonify 5 minutes. Spin 10 seconds.
3. wash tip with 2 x 100 µl ACN (draw 100 µl of solvent up and release; slowly. Do it twice.)
4. wash tip with 3 x 100 µl 0.1% TFA
5. draw sample up and down without air in 10 slow repetitions
6. wash tip with 2 x 100 µl 0.1 % TFA

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At this step you can conveniently store the tip-bound peptides. Put the tip into the eppendorf with remaining 0.1% TFA at 4°C. The peptides are good for at least two weeks without deterioration. Dried out tips can be eluted almost just as fine.

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7. elute peptides with 2 x 100 µl 70 % ACN 1% FA or 70% ACN 0.1% TFA. Each time draw slowly up and down 5 times.
  8. dry the eluate in a vacuum concentrator
  9. add 4-20 µl 5 % FA or 0.1 % TFA, vortex, spin 5 seconds and sonify 5 minutes. Spin again 5 seconds
  10. transfer to MS facility

Steps 9 and 10 depend on what solvent and volume is required for the subsequent analysis, standard is 2% FA in 10-15 µl.

#### Solution stocks:

0.1 % TFA

Rinse a 100 ml bottle twice with water. Mix 400 µl 25 % TFA with 100 ml water directly in the bottle. Store at room temperature. Prepare new if you see particles in the solution.

70 % ACN 1% FA

Rinse a 100 ml bottle and a 25 ml measuring cylinder once with water and once with ~ 5 ml ACN. In the glass bottle mix 2 x 17.5 ml ACN, 14.5 ml water and 500 µl FA. Store at room temperature. Prepare new if you see particles in the solution.

100 % ACN

Rinse a 100 ml bottle twice with ~ 10 ml ACN and fill with 50 ml ACN. Store at room temperature. Prepare new if you see particles in the liquid.