

Desalting of gel-extracted peptides with 10 µl ZipTips

ZipTips are pipet tips with a small block of C18 reversed phase material at the very end. They are used just by drawing solutions up and down without air. A P10 tip can bind ~ 5 µg peptides (upon saturation, please check vendor information). This corresponds to a strong but not bulging single-lane coomassie band.

For desalting larger amounts please use 100 µl tips

Materials:

10 µl C18 ZipTip
20 µl pipet
low-bind Eppendorf tubes
Racks, mini centrifuge.

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. dissolve in 10 µl 0.1 % TFA (sonicate for 5 mins, spin down)
3. wash tip with 2 x 20 µl ACN (draw 20 µl of solvent up and release; slowly. Do it twice.)
4. wash tip with 3 x 20 µl 0.1% TFA
5. draw sample up and down without air in 10 slow repetitions
6. wash tip with 2 x 20 µl 0.1 % TFA

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 10 µl 70 % ACN 1% FA or 70% ACN 0.1% TFA. Each time draw slow up and down at least 5 times.
 8. dry the eluate in a vacuum concentrator
 9. add 4-20 µl 2 % FA, vortex, spin 5 seconds and sonify 5 minutes. Spin again 5 seconds
 10. Bring them to Mass Spec facility

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

Solution stocks:

0.1 % TFA

Rinse a 100 ml bottle twice with water. Mix 100 µl 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.