Desalting of tryptic peptide mixtures using C18 Zip-tips

This step is needed only for the direct analysis of tryptic peptide mixtures by MALDI mass spectrometry. If the sample will be prepared for MS analysis using a nano-HPLC or submitted to LC-ESI mass spectrometry, this step can be omitted.

Materials
Trifluoroacetic acid (Fluka)
Zip-tips, C18 (Millipore)

1. Dissolve dried peptide mixtures (obtained from steps 14 through 19) in 10 µl of 5 % acetonitrile containing 0.1% trifluoroacetic acid.

   For not completely dried peptide mixtures, add ultrapure water to make a volume of 8.5 µl, and add 0.5 µl acetonitrile and 1 µl of 1% trifluoroacetic acid.

   Check pH of resulting peptide mixture by using a pH paper; confirm and eventually adjust pH of samples to 2-4 using 1 % trifluoroacetic acid.

2. Prepare Zip-tip C18 (Millipore).
   Activate Zip-tip by rinsing 3 times with 10 µl acetonitrile.
   Equilibrate Zip-tip 3 times with 10 µl of 5 % acetonitrile containing 0.1 % trifluoroacetic acid.

3. Bind the peptides by aspirating and dispensing peptide solution; use up to 10 cycles.

4. Wash with 10 µl of 5 % acetonitrile containing 0.1% trifluoroacetic acid.

5. Elute peptides with 10 µl of 50 % acetonitrile containing 0.1 % trifluoroacetic acid. Carefully, aspirate and dispense eluant through Zip-tip without introducing air; repeat up to10 times.

   Alternatively, elute the digested peptide with 10 µl of 20% acetonitrile containing 0.1% trifluoroacetic acid and with 10 µl of 60% acetonitrile containing 0.1% trifluoroacetic acid. Combine these two eluants.

6. Dry eluant using a Speed-Vac centrifuge at 35 °C.

7. If necessary, store at -20 °C (< 24 hr) or -80 °C (for longer periods).

8. Prepare sample for MALDI mass spectrometric analysis:

   Resolubilize the desalted peptide lyophilate with 1 µL matrix solution (10 µg/µL α-cyano-4-hydroxy-cinnamic acid in 0.1% trifluoroacetic acid:acetonitrile, 1:1, v/v).

   Load a half microliter of sample mixture on a MALDI target plate (with 144 spots for the AB 4700 instrument) and let dry at room temperature. The sample is now ready for MALDI mass spectrometry.