SAMPLE DESALTING PROCEDURE

Protein samples preparation and storage is done in buffers. These buffer solutions contain salts that form adducts with proteins, thus complicating data interpretation. These non- volatile salts also suppress ionization during MS analysis. Therefore, sample desalting procedure is a crucial step prior to introduction into the mass spectrometer system.

The procedure is as follows:

1. Add 50% acetonitrile (ACN) to the C18 spin column; gently tap the column... Close the top and bottom lid... Give a gentle spin (low RPM... 4000-5000). Allow to settle down...
2. Remove the bottom and top lid... add 50% ACN again followed by gentle spin... Repeat it twice...
3. At this stage resin gets activated ... **[Never leave the resin dry during any of the process]**
4. Now add 100% ACN/0.1% formic acid or 0.1% TFA (single solution) ... spin gently...
5. Discard solution and repeat it 3-4 times...
6. Equilibrate the column with 0.1% formic acid or 0.1% TFA. Add 200 µL of 0.1% formic acid or 0.1% TFA... Gently spin... discard... repeat it 3-4 times...
7. Now add the reconstituted sample **[Before adding the sample to the spin column, give high speed spin 12,000 RPM for 5 minutes... make sure there are no un-dissolved or particulate materials; carefully take out the supernatant alone, and store the un-dissolved pellet at -20°C]**
8. Add the supernatant to the column, gently spin (very low 800-1000 RPM); Collect the flow-through; add it again to the column... repeat this step 2-3 times **[This is to ensure maximum binding]**.
9. Now wash the column with 0.1% formic acid or 0.1% TFA (100-200 µL), collect the flowthrough and freeze it at -20°C.
10. Elute the bound peptide with 40-50% ACN/0.1% formic acid or 0.1% TFA (75 µL)... collect the eluent... Repeat it once more... Pool the collected eluent... Label it as Eluent 01 (E1)
11. Final wash with 100% ACN/0.1% formic acid or 0.1% TFA.
12. Now add 50% ACN to the spin column, pass this solution twice... close the bottom and top lid... store the resin wet in 200 µL of 50% ACN at RT
13. Vacuum dry the eluent (E1) at RT (speed vacuum) ... after complete vacuum dry, you will find very fine dots in eluents...
14. After vacuum dry, store the samples at -20°C or can be used for LC-MS analysis.