

Desalting Protocol for MS

Necessary reagents and instrumentation:

Acetonitrile (ACN)

Trifluoroacetic acid (TFA)

Heptofluorobutyric acid (HFBA)

Cartridge: Ultramicro Spin (www.nestgrp.com) . Mini columns C18, Part # SS18V

Protocol

Max protein capacity 5 to 50 ug

Dilute samples to 5% ACN: 0.1% TFA in H₂O

1. Saturate column with ACN using solvent A (80% ACN, 0.1% TFA). Add 100 ul and centrifuge 4 min at 3100 rpm (repeat)
2. Equilibrate column with 100 ul of solvent B (5% ACN, 0.1% TFA). Centrifuge 4 min at 3100 rpm (repeat twice)
3. Blot dry tip of column and change collecting tube.
4. Add up to 50 ug (max vol 100 ul) of protein digest per column. Collect flow-through
5. Wash column with 100 ul of solvent B (repeat twice)
6. Change collecting tube and collect clean desalted sample with 50 or 100 ul of solvent A
7. Speed vac dry in a glass tube
8. Resuspend in 0.1% Formic acid to final concentration of 1ug/ul
9. Dilute 1:100 with 5% ACN, 0.05% heptofluorobutyric acid (HFBA), 0.4% HoAC