

C18 CapTrap Cleanup Protocol – Mobley Lab

Following Digestion it is necessary to purify a peptide sample by a reverse phase column prior to running it on the mass spec.

This can be accomplished by a vented trap column in-line with the regular column on the instrument, or it can be accomplished off line using a cap trap.

If the later route is applied then it is necessary to use the appropriate capacity trap. The Microm captraps hold 2 µg of sample, the microtraps hold 20µg, and the macrotraps hold 200µg.

For in-solution digestions using Urea or AmBic a 10% solution of formic acid is added to a final concentration of 1%, and the trap is conditioned and loaded using a syringe pump as follows.

- 1) Wash with x µl of 70% acetonitrile containing 0.1% formic acid.
- 2) Equilibrate with the same volume of 0.1% formic acid.
- 3) Load the sample and wash again as in #2.
- 4) Elute with y µL of 70% acetonitrile containing 0.1% formic acid.
- 5) Recondition and wash the column using x µL of 50% acetonitrile without acid.
- 6) Store trap in a eppendorf tube in MeOH and indicate the direction of flow.

Volumes; Speed per 30 seconds and syringe volume equate to x.

captrap x= 100µl, y= 25µl;

microtrap x=250µl, y= 100µl;

macrotrap x= 1ml, y= 250µl.