Proteomics, Metabolomics and Mass Spectrometry Facility
Department of Chemistry and Biochemistry
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Filter –Aided Sample Preparation for nano-LCMS analysis

1- Rinse Nanosep molecular weight cut-off spin filter (3K) with 200 uL of 50 mM Amm. Bicar. pH 7.5 and centrifuge at 5K rpm for 5 min (2x).
2- After collecting the purified proteins (beads: Pull-downs, SEC fraction …) place the material onto the spin filter and exchange the buffer using the buffer mentioned in the first step.
   - **CAUTION**: it is important to eliminate incompatible detergents like Triton, Tween, SDS, and PEG. It is recommended to perform the digestion in Amm. Bicar. pH 7.5.
3- Repeat the second step multiple times until all incompatible detergents/salts are exchanged.
4- Add 10 uL of 100 mM DTT solution to your sample and incubate at RT for 30 min.
5- Wash the sample by adding the 200 uL of Amm. Bicarb. and centrifuge at 5K rpm for 5 min (centrifugation time may increase up to 15 min depending on the sample. Centrifugation speed may increase to 8K rpm).
6- Add 10 uL 100 mM IAM solution to the sample and incubate in the dark at RT for 20 min.
7- Repeat step number five.
8- Add sequencing grade trypsin at a ratio of 1:50 trypsin to total protein after adjusting to volume to 50 uL of Amm. Bicarb. and incubate at 37C for 8-16 hours.
9- Spin the sample on the next day to collect the tryptic peptides (volume should be around 50 uL.
10- SpeedVac the collected peptides to concentrate the sample for 20 min.
11- Vortex the sample for 1 min.
12- Centrifuge the sample at 15 K for 10 min.
13- Transfer only 20 uL of the sample to mass spec sample vial and submit it to the mass spec facility.

If you have questions on the sample preparation and availability of reagents feel free to email the facility manager ***ganesh@montana.edu***