Soluble gel digestion

Developed in the Saito Lab and adapted by Noelle Held.

Materials

Make everything in LCMS grade H2O, use only LCMS grade reagents * organic precipitated protein samples * 100mM Ammonium bicarb (Ambic 100) * Urea * DTT

Prep

- Make fresh 7.5M urea in Ambic 100 (2.25g/5mL)
- make 200mM DTT in ambic 100 (30.9mg/mL)
- make 200mM IODA in ambic 100 (37mg/mL)

Protocol

- To protein pellets add 100uL urea + 15uL ambic 100 (final concentration is about 6M urea)
- Incubate RT 15min, vortex periodically
- Incubate 5min 95C to fully resuspend
- Take 100uL aliquots and safe remainder
- To aliquots, add 5uL 200mM DTT, incubate 56C 400rPM 1 hr
- Vortex and spin down
- Add 20uL 200mM IODA, incubate 1hr with shaking RT in the dark
- Meanwhile reconstitute 100ug trypsin on ice in 0.5mL 50mM acetic acid for 30min with periodic mixing
- Add trypsin in 1:50 ratio with the amount of protein in the aliquot as determined by BCA assay
- Incubate 37C overnight with shaking
- vortex, spin down samples and collect the top. A pellet may occur
- Speed vac to desired concentration (typically 1ug/uL)
- Acidify to pH 4 with acetic acid
- If urea precipitation is a problem, zip tip the sample
- Dilute in buffer B if needed to desired concentration