In-solution digestion

<Notice!> Wearing cleaned gloves is needed, and manipulating in laminar flow cabinet is preferred.

Prepare reagents before use:

- ✓ 50 mM ammonium bicarbonate (ABC) solution (pH 8.5)
- ✓ 12.5 ng/μL trypsin stock

Procedure

1. Add 50 μL of 50 mM ABC solution to redissolve proteins from acetone precipitation.
2. Add 48 mg of urea into sample. Final concentration of urea is 8M and the volume is ~100 ul.
3. Add 1 ul of 1 M DTT. Incubate at RT for 30 min.
4. Add 5 ul of 1 M IAM. Incubate at RT for 30 min.
5. Add 400 ul of 50 mM ABC
6. Add trypsin (w/w, enzyme: protein=1: 20~50) and incubate at 37℃ for at least 16 hr.
7. Perform stage tip desalting.
8. Vacuum dry the sample.
9. Add 0.1% formic acid.