Acetone precipitation of proteins

Reagents & Solutions

- Ice-cold Acetone, HPLC grade (-20°C) *

Equipment

- Benchtop Eppendorf centrifuge *

* available in the core facility

Protocol

1. Add 4x volume of ice-cold acetone to the protein sample[1]
2. Incubate for 15 min at -80°C, followed by 90-120 min incubation at -20°C[2]
3. Centrifuge the tubes for 15 min at 16.000 × g
4. Carefully remove the supernatant[3]
5. Add 500 µL ice-cold acetone to the protein pellet
6. Centrifuge for 5 min at 16.000 × g, discard supernatant
7. Repeat this step one more time
8. Air-dry pellet and resuspend in buffer of choice, depending on the digestion protocol

Notes

[1] Use 2 mL tubes for sample volumes larger than 250 µL. Use 5 mL tubes for sample volumes larger than 350 µL. Use 10 mL falcon tubes for samples larger than 900 µL.

[2] Precipitation overnight is also possible, but not necessary.

[3] In case of low amounts of proteins it can happen that the pellet is not clearly visible. Add GlycoBlue co-precipitant to increase visibility of the proteins.